Assessment of genetic diversity and population structure of Zambian common bean landraces

Abaca, Alex

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A thesis submitted for the degree of Doctor of Philosophy

University of Bath

Department of Biology and Biochemistry

August 2018

Signed

Alex Abaca

Acknowledgement

First and foremost, I would like to extend my sincere thanks to the Almighty God for His protection, guidance and support during my entire academic life journey up to this time. In the same way, I am very grateful to my supervisors Dr. John R Beeching and Professor Rod Scott for their endless support, guidance, encouragement, and intellectual direction that has allowed me to move towards the completion of this thesis.

My sincere gratitude further goes to my funders: the University of Bath through the 50th Anniversary Excellence Scholarships for International Student, Crop Innovations and Self Help Africa for providing Stipends and additional research funding respectively. I would like to appreciate the financial contributions from Bath Alumni Office, Royal Commonwealth Society of Bath, and the Santander mobility Awards that supported me entirely during my field work in Zambia.

Sincere thanks further goes to the Faculty of Science graduate office and the graduate school advisory team for their tireless support right from induction week through provision of postgraduate skills training to thesis preparation and submission for examination. Special thanks to Simon Gane for the graduate coordination and Financial advise that he offered to me throughout my PhD journey.

In conducting my field work in Zambia, I would like to extend my sincere appreciation to Self Help Africa (SHA) Zambia Office that provided work space and transport during my field work; the staff of Zambian Agricultural Research Institute (ZARI) that provided details about the common bean landraces being investigated and soil characterisation; the staff of Faculty of Agriculture at University of Zambia (UNZA) for sharing their bean breeding objectives and challenges and soils characterisation of Zambia; individual farmers under the Shimabala and Mthilakubili seed growers associations that shared extensively their common bean farming practices in Kafue and Lundazi districts respectively; the Zambian Meteorological Department (ZMD) that provided the climatic data and Seed Co seed company. Some names are worth mentioning particularly Elia Manda and Mate Albert of SHA, Kennedy Muimui, Lungu Robert and Raby Banda of ZARI, Kelvin Kamfwa and Isaac O Lungu of UNZA, and Edson Kakonde of ZMD amongst others. Thank you sincerely.

Special thanks to my past and current PhD students who supported me generously Dr. Ahmad Fathoni, Dr. Liu Shi, Dr. Lydia Alhalaseh, Dr. Glenda Orlegde, Toby Parks, and Yi-Wen Lim; the plant group team Dr. James Doughty, Dr. Volkan Cevic, Dr. BaoXiu Qi, the data analysis advisory team Professor Mathew Wills, Dr. Daniel Henk, Dr. Daniel Falush, Dr Sergio Martinez, and Dr. Christopher Hallsworth, and the glasshouse technicians James and Rob.

Finally, I would like thank the contribution of my family members for their guidance and support during my PhD research journey. My brave children who have lived without me for all this long and they have never forgotten to call me daddy. I can't mention all your names but you remain my motivation to even achieve more, and you are my heroes. Thank you.

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List of Abbreviations

AAS – Atomic Absorption Spectroscopy

ADP - Andean diversity panel

AEA - average energy allowance

AFLP - amplified fragment length polymorphism

ARA – Acetylene reductase activity

BAC – Bacterial artificial chromosome

CA - Conservation Agriculture

CBC – Congurity backcrossing

CIAT - Centre for International Tropical Agriculture

cm - centimetres

CT - Conventional Tillage

CTAB – Cetyltrymethyl Ammonium bromide

CWRs - Crops wild relatives

DA - Descriptive analysis

DoA - Department of Agriculture

DRC - Democratic Republic of Congo

ENSO - El Nino/Southern Oscillation

ESADDI - estimated safe and adequate daily dietary intakes

EST-SSR - expressed sequence tag

FAO - Food and Agricultural Organisations of the United Nations

FGD - Focus Group Discussion

FoDiS - food crop diversification support project

GBS – Genotypying by sequencing

GD – Group discussion

GWAS - Genome wide association study

Ha - hectares

IDA - iron deficiency anaemia

iPBS – inter-primer binding site

IRAP – Inter-retrotransposon amplified polymorphism

ISSD – Integrated seed sector development

ITCZ - Inter-tropical convergence zone () as well as the

Kg –– Kilograms

LRR – leucine-rich repeat

masl - metres above sea level

MDP - Middle/Meso American Diversity Panel

mg - milligram

mm - millimetres

MNDs - mineral nutrients deficiencies

MR - minimum requirement

NARIs - National Agricultural Research Institutes

NARS – National Agricultural research system

NGO - Non Governmental Organisation

NGS – Next generation sequencing

PABRA - Pan Africa Beans Research Alliance

PCR - polymerase chain reaction

pH - measure of acidity or alkalinity

PPB - Participatory Plant Breeding

PVS – participatory variety selection

QTL - quantitative trait loci

RAD – Restriction associated DNA

RAPD - random amplified polymorphic DNA

RDA - recommended dietary allowance

RFLP - restriction fragment length polymorphism

RH – relative humidity

SABRN - Southern African Bean research Network

SCN - soybean cyst nematode

SHA - Self Help Africa

SNPs – Single nucleotide polymorphisms

SSA - Sub-Saharan Africa

SSAP – Sequence specific amplified polymorphism

SSCP – single-stranded conformation polymorphism

SSRs - single sequence repeats

STSs - sequence-tagged sites

T - Tonnes

TBAS - traditional based agricultural systems

TEs - Transposable elements

UNZA - University of Zambia

VAD - Vitamin A deficiency

WCRs - wild crop relatives

ZARI - Zambia Agricultural Research Institute

ZMD - Zambian Meteorological Department

Abstract

Analysis of relationships among common bean landrace populations through genetic diversity and population structure using both molecular and agro-morphological methods and their comparisons provide complementary information for crop improvement, conservation and landrace registration programmes. However, such information is not available for Zambian common bean landraces. To fill this gap, thirty agro-morphological traits and 20 SSR markers were used on 124 individuals and on over 1100 individuals of four landraces to study agro-morphological and genetic diversity respectively among these landraces, assess the genetic changes that occur over the three growing seasons, the correlations among the agro-morphological traits, nutritional variations, and classify the accessions into the two genepools based on both markers using reference genotypes from CIAT. The results from these studies showed that there is a very high genetic diversity at molecular (0.605) and agro-morphological (0.404) levels for the landraces, commercial varieties and CIAT reference lines overall. Molecular diversity showed higher values in the landraces than commercial varieties and CIAT reference lines (0.517 vs 0.253). The same was true for agro-morphological diversity (0.469 vs 0.146). At molecular level, there was a high rate of gene flow amongst these landraces (Nm = 0.3906). There was no significant differences ($p < 0.05$) in genetic diversity and population structure among the the three growing seasons for the landraces. Morphological characters showed very strong correlations amongst themselves such as Pod length vs days to flowering, average seed weight per plant vs days to flowering and 100 seed weight to days to maturity. There was a significant ($P < 0.05$) differences in the mineral concentrations between the landraces, commercial varieties and CIAT reference lines. Twenty three and twenty six sub-populations with iron and zinc content higher than averages respectively were identified. There was no significant difference ($p<0.05$) for mineral concentration based on seed colour although yellow, red, maroon, and black seed colours were associasted with high mineral contents. A strong positive correlations were exhibited among the mineral concentrations, for example Zn vs Fe ($r = 0.764$). The four landraces were identified as Lusaka yellow and Lundazi being mostly Andean beans, and Mbala mixture and Solwezi being mostly Mesoamerican beans. Overall, Mesoamerican beans (54%) dominate these common bean landraces from Zambia. These results demonstrate a lot of practical importance in maintaining genetic diversity and biofortification that can be achieved through incorporating these landraces in to the Zambian bean breeding program, in addition to facilitating landrace registration, and landrace seed conservation.

Chapter 1

General Introduction

1.1 The common bean

The domesticated common bean (*Phaseolus vulgaris* L.) also referred to as dry bean, was originally a crop of the New World (Beccera *et al*., 2010; Blair *et al*., 2012), but it is now grown and consumed extensively, and has remained an important crop in all major continental areas in Africa, Europe, South and North America, Australia, and Asia (Burle *et al*., 2010; Angioi *et al*., 2011). It is the most important source of dietary protein, vitamins, minerals, and fibre, and third most important source of calories for African and Latin American households with lower income after cassava and maize (Beebe *et al*., 2000a; Broughton *et al*., 2003; Wang *et al*., 2012). Its protein is high in lysine, which is relatively deficient in maize, cassava and rice, making it a good complement to these staples in the diet in many developing countries (Baudoin and Maquet, 1999). The minerals in beans are readily available particularly calcium, magnesium, iron and zinc and their roles in fighting deficiencies and reducing other risks such as osteoporosis and hypertension have been explained (Akond *et al*., 2011). Regular consumption of common bean and other pulses is now promoted by health organizations because it reduces the risk of diseases such as cancer, diabetes or coronary heart diseases (Bennink, 2002), because common bean is low in fat and is cholesterol free. Based on the part that is edible, common bean can be categorised as dry beans (dry seeds) or snap beans (green pods) and are widely accepted in major national and international markets; whereas, based on the seed types common beans can be categorised as landraces (primitive/traditional garden form) or commercial or improved varieties (resulted from genetic improvement of the breeding programme) (Zeven, 1999). In Africa, production of beans is dominated by dry beans with both landraces and commercial varieties being evident in the production systems. Common bean plays an important role in the soil fertility amendment practices of low input farming systems of Africa (Jansen *et al*., 2007), and the crop has received appreciation throughout the Eastern and Southern Africa because they have a long storage life, good nutritional properties, low cost compared to animal proteins, and can be easily stored and prepared for eating (Katungi *et al*., 2009). Besides its importance in food, health and nutritional security, soil fertility, beans provide a steady and lucrative source of income for many rural households through the sales of surplus harvest (FAOSTAT, 2011).

1.2 Common bean and its production environment

Common bean is widely adapted to environmental conditions (Figure 1.1): annual rainfall amounts of 500–2000 mm, not sensitive to soil types (clay, sandy, loam, volcanic or alluvial), altitude of near sea level in the continental USA and Europe to elevations of more than 3000 m in Andean South America and East African highland areas, mean temperature of 16-24^{O}C, 52°N to 32°S latitude and a varying range of pH of 3.2 in Mbala district of Zambia to 6.8 in other parts of Africa (CIAT 1989; van Schoonhoven and Voysest, 1991; Wortmann *et al*., 1998; Katungi *et al.*, 2009). Common bean is considered a short season crop with most varieties maturing in the range of 65 to 150 days from emergence to physiological maturing (Buruchara, 2007; CIAT, 1989). The crop is a warm season crop that does not tolerate frost or long periods of exposure to near freezing temperatures at any stage of growth. In Africa, crop cultivation is concentrated at altitudes above 1000 metres above sea level (masl), with adequate amounts of precipitation (> 400 mm of rain) during crop growing season and soil pH above 5.5 (CIAT 1989; Wortmann *et al*., 1998). This wide variability of production environments results in a massive diversity of the cropping system, plant types and production constraints (CIAT, 1989). These factors, coupled with highly specific local preferences for particular seed types or colours have complicated attempts for bean improvement programme by the different breeding programmes.

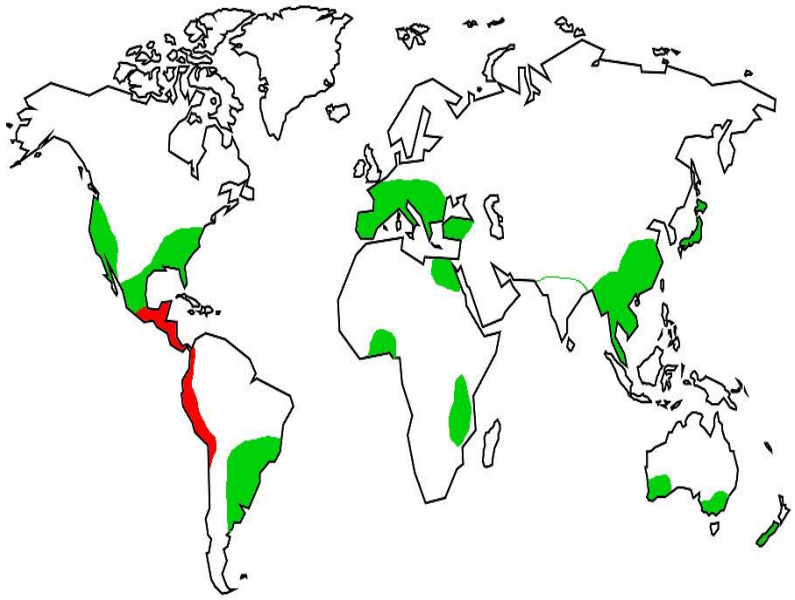
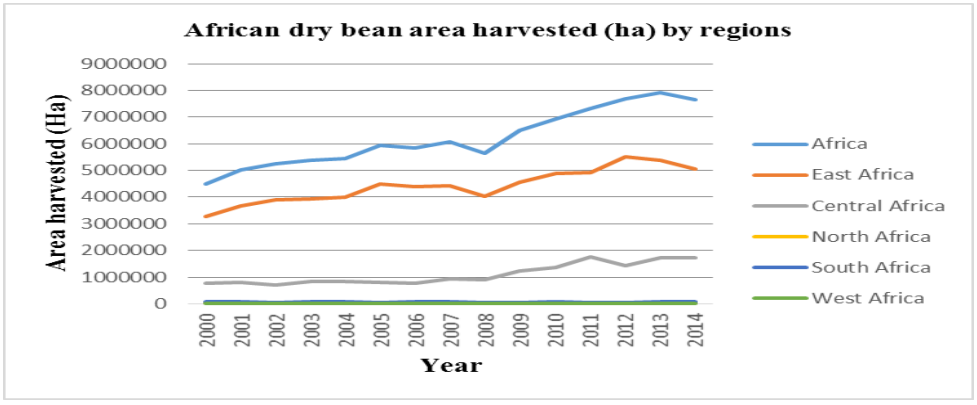
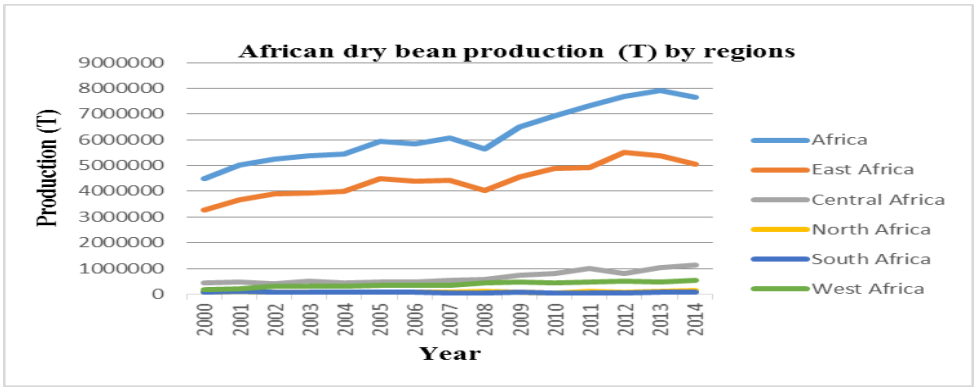


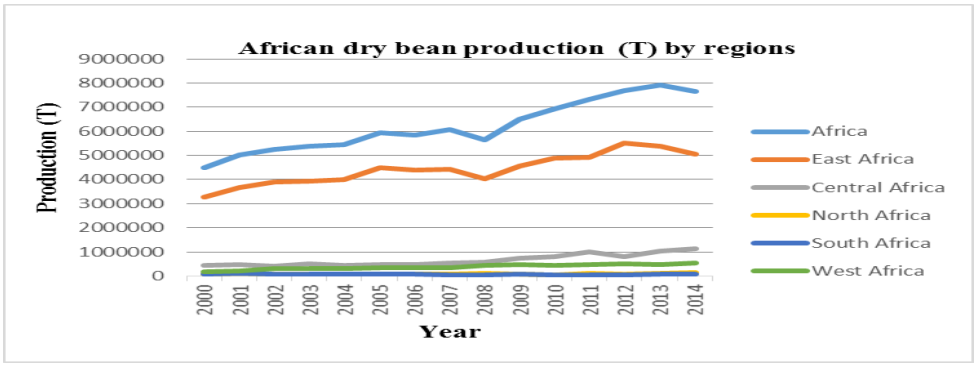
Figure 1.1 Origin and Distribution of Common bean**. Green** represents major producing areas and **Red** represent the centre of origin. Adapted from Burcher *et al*., 1988.

1.3 Common bean in Africa

Common bean is an important grain legume grown on over 4 million of hectares every year in Eastern, Central and Southern Africa (Figure 1.2) (FAOSTAT, 2017) where bean consumption per capita exceeds 50 kg a year and is perhaps the highest in the world, reaching over 66 kg in densely populated Western Kenya (Wortmann *et al*., 1998). Production in Africa is primarily by small-scale farmers, mainly women, with few commercial farms (Kambewa, 1997; David *et al*., 2000; Xavery *et al*., 2005; Monyo and Varshney, 2016). In Eastern and Southern Africa, beans are grown under multiple cropping systems, mainly in association with maize, banana, sugar cane, coffee, roots and tubers, sorghum or millet (Allen and Edje, 1990), with the exception of Ethiopia where white canning beans (which account for about 50 percent of the total) are grown as a sole crop. In Latin America, Asia and Europe production of beans tends to be cantered on smaller holdings although the cropping system used can vary from the highly mechanized, irrigated, and intensive production of monoculture bush beans, to complex associations of indeterminate or climbing beans with corn, other cereals, or plantain (Liebenberg, 2002). The crop yields can range from less than 500 kg ha^{-1} in parts of Latin America and Africa to as much as 5000 kg ha^{-1} under experimental conditions. In Eastern and Southern Africa, common bean is grown twice a year, with sowing seasons running from March to April and from September to October, except in parts of Ethiopia and northern Uganda where the main growing season is June to August (Rukandema, 1981; Wortmann *et al*., 1998). Cultivation of common bean in Africa is widespread in many parts but production of approximately 80 percent is concentrated in Eastern and Southern regions of the continent as summarised in figure 1.2 (Katungi *et al*., 2009; FAOSTAT, 2016). The top ten producers in Africa are Kenya, Uganda, Tanzania, Rwanda, Angola, Burundi, Democratic Republic of Congo (DRC), Malawi, Ethiopia, Madagascar (Katungi *et al*., 2009). It is important to note here that, Zambia as a country in Southern Africa is boarded by 4 of the top 10 producers of common bean in Africa (DRC in the North, Tanzania in the North East, Malawi in the East, and Angola in the West and North-West regions of Zambia), thus the importance of common bean in Zambia can easily be attached to its location, and to its neighbouring countries.







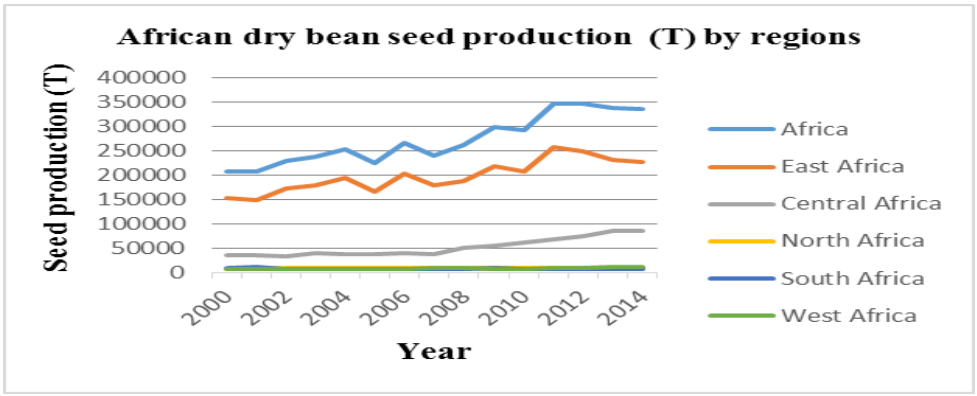


Figure 1.2 Trend in African dry bean production, area harvested and yields by regions. Adopted from FAOSTAT, 2016.

1.5 Common bean in Zambia

Specifically for Zambia, common bean ranks second after groundnuts among the grain legumes in terms of its economic importance, mostly in low income households due to its affordability, and it constitutes about 32.1% of the total area under food legume crops (SCO, 2012 cited in Hamazakaza *et al*., 2014). Zambia's agricultural policies were adjusted and refocused to lay emphasis on crop diversification away from maize dominated to include low-input crops like food legumes (Hamazakaza *et al*., 2014). This has enabled a shift from maize dominated production as a sole crop to maize-legumes intercrops (Siame, Willey, Morse, 1998) and hence diversification of the agricultural production, sources of income and nutrients, and have proven to be more appropriate for resource-poor and small-scale farmers. Chalwe (2011) showed that beans are produced in all the provinces of Zambia, with the top four provinces accounting for 87.2% of bean production (Northern 59.19 percent, Luapula 10.59 percent, Central 9.24 percent, and North Western 8.18 percent). The National Agriculture Research Systems (NARS) of Zambia through Zambia Agricultural Research Institute (ZARI) have released ten improved bean varieties as of 2014, seven of which are bush varieties, two are semi-climbers and one is of climbing type. Despite these bean breeding efforts and diversification in agricultural production, Zambia still remains a net importer of bean grain compared to its neighbours, such as Tanzania and Mozambique, which are net bean exporters (Hamazakaza *et al*., 2014). Common bean production in Zambia is prone to a number of biotic and abiotic stresses that hinder production, including massive use of low yielding varieties, very acidic soils, lack of trusted seed companies, insect infestation, disease and increasingly climate related constraints particularly drought (Katungi *et al*., 2009; Muimui, Kimani and Muthomi, 2011; Muthomi, Muimui, and Kimani, 2011; Hamazakaza *et al*., 2014). The common bean varieties grown in Zambia comprise improved, landraces and varieties imported through cross-border trade with Tanzania, Democratic Republic of Congo (DRC), Angola, Namibia, Zimbabwe and Malawi. A study conducted by Hamazakaza *et al.* (2014) in Muchinga and Northern provinces on the access and adoption of improved common bean varieties in Zambia revealed that, overall and within the provinces studied, *Kabulangeti* local is the most popular variety grown, followed by *Lusaka yellow* and *Mandima* locals. The study further revealed that local varieties are grown across all the wards, and that no geographical clustering seemed to emerge from the two provinces studied. At plot level, the same study reported that 15.87% of bean plots were planted with both local and improved varieties in a mixture.

1.4 Common bean seed systems in Africa

The bean seed systems in Africa can be categorised as public or private, used interchangeably with formal and informal seed systems, respectively (David and Sperling, 1999; Louwaars and De Boef, 2012). The public or formal sector seed programmes in most sub-Saharan African countries that targeted the dissemination of quality seed of improved varieties in the 1970s and 1980s did not take into account the role of private or informal seed programmes and assumed that it (informal seed system) would disappear (Louwaars and De Boef, 2012). Several orientations and adjustments were made in 1990s that shifted towards the withdrawal of the public sector and promoting privatization and liberalization of the seed market, causing the informal seed system to remain dominant (Louwaars and De Boef, 2012). Currently, the public seed program supplies about 20 percent of the required seed (Katungi *et al*., 2009), which is mainly hybrid. Rubyogo *et al*. (2007) noted that in order to properly understand the seed systems in Africa, there is need to understand the clientele to be served who are mostly small scale farmers with poor resources and more particularly women, and the various production and marketing constraints that they face. This understanding of the clientele would promote seed security, defined as 'the state in which a farmer has access to the sufficient quantities of seeds of their preferred varieties with adequate physical quality, at the right time of planting' (Sperling and Cooper, 2004). This was realised after the work of David and Sperling (1999) based on seed systems research in Uganda, Rwanda, Burundi, and the DRC showed that the four commonly held basic assumptions that: small-scale farmers do not buy seeds as they mainly rely on their own stocks or obtain from their neighbours; small-scale farmers cannot afford to buy seeds of newly introduced bean varieties or will not risk it; farmer seed system networks function efficiently in varietal diffusion; and a good variety will sell itself, are all false.

Integrated seed sector development (ISSD) that aims at linking informal and formal seed systems was later proposed to balance public and private sector involvement through analysis of strengths and weakness of the local seed systems to make both informal and formal systems to be complementary to each other (Penrose-Buckley, 2007). Penrose-Buckley (2007) further explained that for ISSD to work well, farmers need to be organized in cohesive groups/producer organizations whose membership comprise both female with different productive economic assets and human and/or social capital can strengthen their participation and gains in the integrated seed sector. This mixture in the group allows for the exploration of variation among seed value chain actors, with the aim of making seed programs and policies more coherent with farmers' practices and more effective at reaching food security (Louwaars and De Boef, 2012).

Rubyogo *et al.* (2007) further outlined key steps that can be followed to developed an efficient integrated seed system for the resource poor farmers as engaging the end users in bean participatory variety selection, participatory assessment of existing bean seed channels and testing alternative seed channels, understanding the limitations of local seed systems, addressing the limitations by integrating formal and informal seed systems, farmers' skills and knowledge enhancement, and new bean variety demand creation and their promotion. From the above discussions, many researchers have realised that the two seed systems complement each other and thus should not be used competitively, but rather supplementary, and cut across countries with both landraces and commercial varieties of common bean in production.

1.6 Common bean landraces

Seed companies (government/public, private, and commercial) in developing countries including Zambia, typically supply no more than 20% of seed for most food crops with crops affected most being: i) self-pollinating crops like beans, groundnuts/peanuts, and rice, ii) vegetatively propagated crops such as cassava, sweet potatoes, yams, and potatoes, and iii) crops with limited demand that include indigenous vegetables, forages, and open pollinated maize varieties (Soniia, 2004; Croomwell and Wiggins, 1993; Grossman *et al*., 1991). Therefore, farmers that are predominantly involved in growing these classes of crops above are responsible for saving their own seeds and/or planting materials for the next season (genetic conservation of landraces). Landraces are crop materials that are traditionally maintained and grown by farmers (Soleri and Cleveland, 2004). Landraces generally share the following characteristics; distinct but variable populations, which usually have a common name, lack 'formal' crop improvement, characterised by a specific adaptation to the environmental conditions of the area of cultivation and are closely associated with the user, knowledge, habits, dialects, and celebration of the people that developed and continue to grow it (Negri, and Tosti, 2002; Negri and Polegri, 2009; Negri *et al*., 2010; Polegri and Negri, 2010).

Landraces (farmer/local varieties) continue to be grown today by small scale farmers in traditionally based agricultural systems (TBAS) including Zambia, allowing for both local and regional consumption needs, and the large social need for the conservation of genetic diversity (Smale, 2002; Zizumbo-villarreal *et al*., 2005). TBAS are characterised by marginal growing environments (biotic and abiotic stresses), continued use of landraces even when the improved/modern crop varieties are available (Brush *et al*., 1992), and growing mixed seeds and/or grains of landraces together during the cultivation process (Zizumbo-villarreal *et al*.,

2005). Farmers' seed management and choice of growing environments under the TBAS determine the possible extent of pollen flow between populations or farmer varieties (Soleri and Cleveland, 2004 and 2009). In terms of seeking potential response of these mixed seeds/grains planted together, farmers may practice intentional selection either to create new varieties, which is best documented in vegetatively propagated and self-pollinating crops or to conserve the existing varieties (Soniia, 2004; Soleri and Cleveland, 2004 and 2009).

A low rate of adoption of modern/improved crop varieties by small scale farmers in developing countries has been noticed (Soniia, 2004; Hamazakaza *et al*., 2014). This has been attributed to factors such as inability of the formal seed system to penetrate the rural farming communities or failure of the centralised seed production system to meet their complex and diverse seed requirements. To address this, farmer seed enterprises have been proposed in many developing countries with the objectives of improving dissemination of modern/improved varieties, preserving genetic diversity and quality, improving seed (local or modern) availability (time, place and quantity), and reducing the cost of seeds and dependence on external seed sources. In addition to farmer seed enterprises, participatory plant breeding of common bean and major crops is being advanced to bring the different actors along the value chain together (Asfaw *et al*., 2012).

1.7 Importance of common bean landraces in the bean improvement program

Many studies that involved the use of common bean landraces have shown their importance in the improvement of common bean as a crop for the future. For example, Chen *et al*. (2014) developed and mapped 90 microsatellite loci from the Chinese common bean landraces based on genome sequences and used them for common bean diversity study; Sousa *et al*. (2015) characterised and mapped the Anthracnose resistance in the Andean beans using a landrace called Corinthiano; Schmutz *et al*. (2014) used both wild and landrace accessions of common bean to assemble the common bean reference genome and later conducted a genome-wide analysis to confirm the dual domestication of common bean; Munoz-Perea *et al*. (2006) identified sources of drought resistance in common bean in Idaho, USA from landraces with different growth habits; Miklas *et al*. (2003) derived a major QTL for common bacterial blight resistance from the common bean great northern landrace cultivar (Montana No. 5); Gonçalves-Vidigal *et al*. (2009) identified an Andean landrace (Jalo Listras Pretas) as the new source of Andean resistance gene to Anthracnose; and Gonçalves-Vidigal *et al*. (2011) mapped the tightly linked genes *Phg-1* and *Co-1* that confer resistance to angular leaf spot and anthracnose in the common bean landraces,

among several studies. Gonçalves-Vidigal *et al*. (2009) observed that there is need to characterised more of the Anthracnose resistance gene for common bean due to resistance break down exhibited by some of the races of Anthracnose disease causative organism (*Colletotrichum lindemuthianum*). This, therefore, implies that common bean breeders will continue to seek the services of common bean landraces to improve commercial varieties. To enable these landraces to be utilised continually, they need to be characterised and their genetic diversity determined. Landrace characterisation and genetic diversity assessment still remain a big challenge when it comes to landraces of African origin due to the fragmentation and isolation of the locations where they are grown that make their collection very difficult. Unreliable climatic conditions such as drought and flood, in addition to infertile soils, low soil pH or soil toxicity, make it difficult to guarantee the existence of these landraces in Africa for future usage. Therefore, there is need to characterise, and assess the diversity parameters of the African common bean landraces using a combination of approaches so as to pave the way for their conservation and their utilisation in the national breeding programs of their respective countries, including Zambia. As mentioned early, genetic diversity of farmer varieties and/or landraces in Africa is not well understood/documented, and yet landraces have been shown to support broad resistance to multiple biotic and abiotic stresses, making them not only valuable to farmers under the traditional based agricultural systems (TBAS), but also to plant breeders and conservationists for future production in industrial agriculture (Ceccarelli *et al*., 2000). The characterisation and identification of African common bean landraces from Zambia remain the bulk of the work that has been reported in this thesis.

1.8 Landrace characterisation and population diversity assessment

Very few studies have characterised common bean of African origin using a combination of molecular and morphological approaches (Asfaw *et al*., 2009; Blair *et al*., 2010; Okii *et al*., 2014a and b), with only one study being dedicated to the landraces of common bean from the East African highland regions of Kenya and Ethiopia (Asfaw *et al*., 2009). Generally, landraces of common bean can be characterised and their genetic diversity assessed using Morphological (quantitative and qualitative parameters), Biochemical (phaseolin seed protein and isozymes) and Molecular approaches such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), single sequence repeats (SSRs), expressed sequence tags (EST-SSRs). Due to the fact that some of the molecular techniques work better for certain crops than for others and that morphological approaches are affected by environmental variations, several studies have assessed the population

diversity of common bean using a combination of approaches. Chiorato *et al*. (2006) used agro-morphological and molecular data to study identification of common bean duplicates; Ligarreto, Gustavo, and Martínez (2014) identified the variability of a common bean collection through morphological, physiological, biochemical, and molecular relationships; Cabral *et al*. (2010) quantified diversity among common bean accessions using Ward-MLM strategy, amongst others. As a result of these observations, in this study of the landraces from Zambia, molecular (using fluorescent labelled single sequence repeats markers), Biochemical (macro and micro element content), and Agro-morphological (quantitative and qualitative) approaches were used.

1.9 Objectives of the study

The broad objective of this study is to characterise and identify common bean landraces from Zambia using a combination of approaches based on molecular (genepools), agro-morphological and macro and micro element contents. Specific objectives of the study are as below:

i. To determine the effect of the biophysical factors (soils and climate) and the farming systems on the beans production and the level of genetic diversity across the 4 locations from which these landraces were collected from and maintained in Zambia
ii. To characterise and assess genetic diversity of these landraces using single sequence repeats (SSR)/microsatellite markers
iii. To characterise and asses the genetic diversity of these landraces using agro-morphological characters
iv. To assess the genetic diversity changes that occur in these landraces from 2014 to 2016 seasons by combining microsatellite markers and Agro-morphological data
v. To characterise macro and micro element concentrations in these Zambian landraces using Atomic Absorption Spectroscopy (AAS)
vi. To determine the genetic composition of these landraces based on the two known gene pools of Andean and Mesoamerican beans
vii. To evaluate the potential contributions of the research to the conservation and improvement of common bean in Zambia

Chapter 2

Literature Review

2.1 *Phaseolus vulgaris*: Origins, domestication and botanical description

2.1.1 Origins, domestication, the two genepools, and the current germplasm resources of Phaseolus vulgaris

Scientists accepted the New World as the origin for Common bean (*Phaseolus vulgaris* L, 2n = 22) after more than 7000 years ago of its domestication (Gepts and Debouck, 1991; Graham and Ranalli, 1997) following the archaeological, botanical, historical and linguistic data from the Americas that confirmed it as the centre of origin for common bean. These archaeological discoveries were made in Peru and further remains of seeds, pod fragments, and even the whole plants of the common bean have been recovered, not only in Andes but also in the Central America (Gepts and Debouck, 1991). These archaeological findings share two characteristics in common that: they are all located in dry areas, whether Andes or in Central America, and they include remains of only domesticated common beans. Gepts and Debouck (1991) noted that, in describing these wild populations, three aspects were particularly relevant: the morphological characteristics of the wild forms, geographic distribution of the wild forms, and the genetic relationships between the wild forms and the cultivars. Therefore, it became apparent that the wild common beans are morphologically differentiated according to their geographic regions for instance, Central American (Mesoamerican) beans had shorter raceme peduncles, larger number of flower nodes per raceme, larger flower bracteoles, and smaller seeds than those of the Southern Andes (Andean beans). Historically, Christopher Columbus after his first voyage to the New World saw new fields planted with *'faxone and fabas/fexoes'*, which are the same as *'frejoles or judias'*, Spanish names for common bean (Gepts and Debouck, 1991). Linguistically, Gepts and Debouck (1991) noted that the vocabulary of several native Indian languages includes specific word designing the common bean, which also points to the antiquity of its cultivation in the Americas. In summary, the existence of archaeological, botanical, historical and linguistic data in the Americas and its absence elsewhere represents strong evidence favouring an American origin for the common bean.

After the above earlier establishment of the centres of domestication for common beans in the Andes and Central America, further evidence was provided that utilised both morphological and molecular approaches. Singh (1989) and Singh *et al*. (1991a,) confirmed these centres of

domestication using morphological, agronomic and allozyme patterns; Gepts and Bliss (1986), Gepts *et al*. (1988), Kami *et al*. (1995), Islam *et al*. (2002), and Kwak and Gepts (2009) used the seed protein (Phaseolin); Becerra and Gepts (1994) and Becerra *et al*. (2010) used nuclear RFLP (Restriction fragment length polymorphism) diversity; Vera *et al*. (1999), Palomino *et al*. (2005), and Szilagyi *et al*. (2011) used RAPD (Random amplified polymorphic DNA) diversity; Tohme *et al*. (1996) used AFLP (Amplified fragment length polymorphism) diversity; Yu *et al*. (2000), Gaitan-Solis *et al*. (2002), Grisi *et al*. (2007), Blair *et al*. (2003; 2006a; 2007; 2010; 2012), Angioi *et al*. (2009a, b), and Wang *et al*. (2012) developed and utilised SSR (Simple sequence repeat) diversity; and Kumpatla and Mukhopadhyay (2005) and Blair *et al*. (2011) developed and utilised EST-SSR (Expressed sequence tag microsatellite) markers.

Detailed studies of the wild ancestral bean forms found in the highland regions of Mexico and Andean South America suggested that multiple domestication events occurred in each region (Koenig *et al*., 1990; Koinange and Gepts, 1992), which resulted in the morphological, physiological and genetic changes of the domesticated common bean (Gepts and Debouck, 1991). Morphologically both vegetative and reproductive involving roots, seed types (colour, size and shape), and pod dehiscent were affected; physiologically sensitivity to photoperiod was noticed; and genetically plant growth habit, and reduced genetic variability were changes reported by Gepts and Debouck (1991). An increase in seed size in the Mesoamerican cultivars could have been introduced through introgression from the larger-seeded runner bean (*Phaseolus coccineus*) as suggested by Gepts and Debouck (1991) and Graham and Ranalli (1997).

Following their successful domestication from the two centres of origins, common bean found itself in all the major continents of the world. Several routes have been suggested for this, for instance, Gepts *et al*. (1988) suggests that the smaller seeded Mesoamerican lines followed a route through Mexico and Central America, via the Caribbean and Northern South America to Brazil. Common bean remains found in the southwestern USA are also likely to have been introduced from Mesoamerica, while Paredes and Gepts (1995) report extensive introgression of Central American germplasm into Chile. The Mesoamerican common bean probably arrived in Europe through Spain and Portugal (Iberian Peninsula) in 1506, and the Andean in the same way in 1528, after the exploration of Peru by Pizarro (Graham and Ranalli, 1997; Angioi *et al*., 2010), and they are likely to have spread into Africa during the slave trade and colonial periods, and into the north-eastern USA through immigration.

In line with the above routes, different seed type compositions have been reported in different countries or continents, which is a reflection of genetic origin. Angioi *et al*. (2010) showed that Europe is dominated by the large seeded Andean bean type, China, Brazil, Cuba and Central America are dominated by the small seeded Mesoamerican bean type, and Africa is dominated by these two bean types in an almost equal proportion (Voysest and Dessert, 1991; Asfaw *et al*., 2009; Blair *et al*., 2010; Okii *et al*., 2014b). Voysest and Dessert (1991) noted that following domestication different seed colours are preferred by different countries, with regional variations within come countries. For example, South American countries of Brazil and Venezuela are dominated by black and red small seeded beans, Mexico is dominated with light coloured yellow beans of small to medium seed size, and in in Africa beans are grown as varietal mixture (Voysest and Dessert, 1991). However, the dominant seed colour in Mexico was affected by the US-Mexico free-trade agreement sometimes referred to as North America free trade agreement (NAFTA) (Burfisher *et al*., 1992; Robinson *et al*., 1993; Zahniser and Link, 2002) that dismantled numerous trade barriers and contributed to an expansion in U.S.A agricultural exports and increased the domestic availability of various farm and food products, thus, cheaper industrially produced beans from USA have flooded Mexico ruining the traditional bean farming system and seed types that existed before. The African bean seeds commonly exists as seed admixtures with less seed type preferences than those in other bean producing countries outside Africa. In Zambia, Hamazakaza *et al*. (2014) showed that admixture consists of both the bean landraces and commercial varieties, with landraces dominating the production.

2.1.2 Common bean and its nutritional quality

The crop is grown primarily for its fresh leaves and pods as vegetables, and dry grains with most nutritional properties linked to their high protein content, carbohydrate, vitamins and mineral content (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010; Petry *et al*., 2015; Chavez-Mendoza and Sanchez, 2017). However, the nutritive components and its contribution to human health can be categorised as chemical composition, bioactive compounds, and mineral composition (Table 2.1), with varying ranges for their values depending on the variety, part of the plant eaten, and seed colour (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010). In terms of the minerals, Akond *et al*. (2011) explained the importance of magnesium, calcium, zinc and iron in the diets, and how these minerals are important in reducing the risks of osteoporosis and hypertension in populations that depend on common beans. Chavez-Mendoza and Sanchez (2017) noted that the zinc content of beans is one of the highest among vegetable sources, and nearly equal to that of the dairy products; whereas, Beebe *et al*. (2000b) observed that the iron-

content values vary between genotypes, and from wild and cultivated beans, although wild beans had only a narrow advantage in iron content over cultivated ones.

Table 2.1 Chemical composition, bioactive compounds, and mineral composition of common beans from different studies

Chemical Composition		**Bioactive Compounds**		**Mineral Composition**	
Chemical	**Range (%)**	**Compound**	**Range (mg/100g)**	**Mineral**	**Range (ppm)**
Ash	3.80-4.7	Saponins	2.65-23.48	Boron	8.2-18
Lipids	0.92-2.0	Flavonoids	6.60-115.72	Calcium	1,054-3,152
Proteins	15.0-26.35	Condensed Tannins	2.15-698.4	Copper	7.2-14
Total starch	35.27-39.84	Anthocyanins	0.0-3.75	Iron	34-89
Carbohydrate	51.51-57.19	Isoflavonoids	0.08-1.29	Manganese	10.6-29
Raw fibre	1.77-2.77	Phenolic acids	0.17-0.68	Sodium	8.0-50
Humidity	8.0-11.95	Dietary fibre	5.5-41[a]	Phosphorus	2,988-7,095
		Oligosaccharides	43.1-57.6	Sulphur	1,786-3,078
		Resistant Starch	28-37[a]	Zinc	21-54
		Lectins	1.5-2.3HAU*/g		
		Protease inhibitors	7.9-11.9TIU**/mg		

[a]percentage and not in mg/100g, *hemagglutinating unit (HAU), **trypsin inhibitor units (TIU): **Source:** Modified from Beebe *et al*. (2000b); Petry *et al*. (2015); Chavez-Mendoza and Sanchez (2017).

Specifically, Tryphone and Nchimbi-Msolla (2010) showed that there is wide genetic variation between genotypes in Iron and Zinc content in Tanzanian bean germplasm, and that this mineral content vary between leaves and seeds. A significant high positive correlations are found among several elements, including iron, zinc, sulphur, manganese, and phosphorus, with iron and zinc with the strongest significant correlation across different genotypes ($r = 0.663$, $p < 0.05$) (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010). The implication of these correlations is that some genetic factors for different minerals are co-segregating and that selection for one element (for example, iron) will in fact result in an increase in other elements (such as zinc). This makes selecting plants with high content in these minerals a cheap source of bio-fortification through breeding programmes. Hotz and McClafferty (2007) noted a number of challenges exist during the development and evaluation of bio-fortified staple foods crops including common beans (Figure 2.1) that need to be addressed.

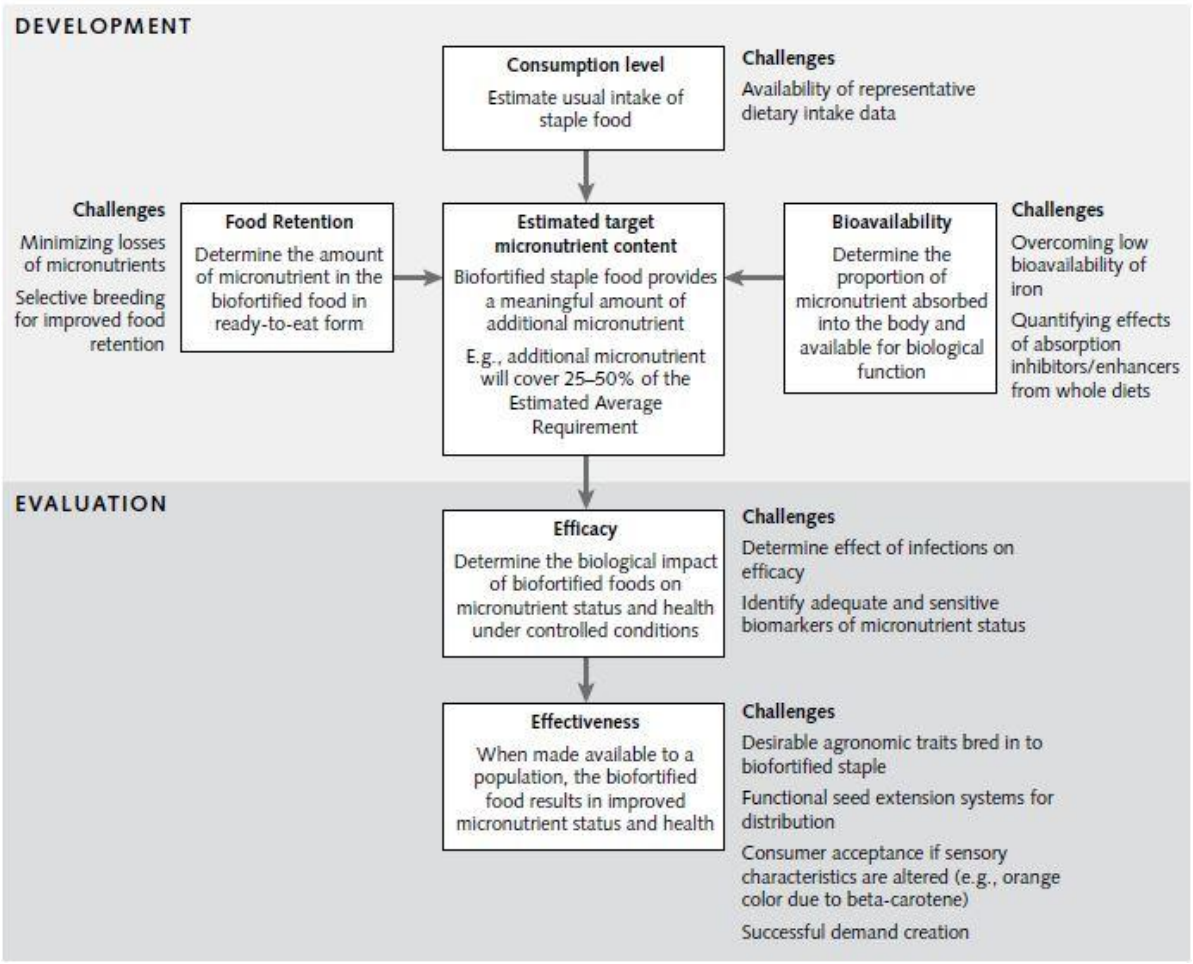


Figure 2.1 Nutritional Research Challenges for Biofortified stable crops during developing and evaluation. Adapted from Hotz and McClafferty, 2007

A review by Chavez-Mendoza and Sanchez (2017) has pointed out that, significant amounts of these chemicals, compounds or minerals are lost at different rates during processing. Rickman *et al*. (2007) reported that vitamins are lost during thermal processing because they are highly sensitive to oxidation, and that the condensed tannins content decreased considerably after the cooking (94%) and frying (95%) processes in the Mexican bean genotypes.

2.1.3 The general botany

The general botany of common bean has been described in the review by Graham and Ranalli (1997). Briefly, *P. vulgaris* is an herbaceous annual member of Leguminosae, tribe Phaseolaea, subfamily Papilionoideae that includes member with both determinate and indeterminate growth habits in cultivation. The plant is initially tap-rooted although adventitious roots then emerge and dominate the tap root. Root architectural variations, and their importance in crop productivity have also been reported (Lynch, 1995), as well as the symbiotic association between roots and

nitrogen fixing bacteria (Hardarson *et al*., 1993; Olivera *et al*., 2004). The flowers are born in auxiliary and terminal raceme, which may contain a single to many flowers. The flower contains 10 stamens and a single multi-ovuled ovary, which is predominantly self-fertilised, and develops into the different levels of pod curvature. The flower colours are genetically independent of the seed colour, but association between particular flower and seed colours is a common phenomenon. Seed description in form of types (colour, shape and size) was provided for by Debouck (1991). These seed descriptions alongside other botanical characters were also used in gene pool classification (Gepts and Debouck, 1991) into Andean and Mesoamerican gene pools. Graham and Ranalli (1997) reported that four growth habits of growth habit I (determinate 'dwarf' plants with 3-7 trifoliate leaves on the main and flowering concentrated over a very short period of time); growth habit II (upright habitat, with an erect stem and branches, often without a guide); growth habit III (bush habit with weak and prostrate stems with numerous branches with a guide); and growth habit IV (mainly climbers if provided with a tutor and with long, weak and twisted stems) as being common among the common bean landraces and varieties in production. These different growth habits respond differently in different Agro-ecologies (Emam *et al*., 2010) and can take on different reproductive adjustments to cope up with the varying levels of challenges in the production environment in order to give adequate yield accordingly.

2.1.4 Reproductive biology

The reproductive unit of *P. vulgaris* occurs in a morphological unit, a compound raceme subtended by axillary flowers and a trifoliate leaf (Sage, 1990), with the auxiliary and basal buds having a higher percentage of flowering than more distal buds on a raceme. *P. vulgaris* is normally self-fertilised, although some exceptions occur in specific tropical locations where outcrossing can be significant. Accordingly, interspecific crossing is rare in nature, though hybridization between common bean and runner bean (*Phaseolus coccineus*) does occur (Thomas *et al*., 1983). A successful cross between tepary bean (*Phaseolus acutifolius*) and common bean, resulted into a cultivar called Great Northern Nebraska 1 selection 27 that carries resistance to common blight caused by *Xanthomonas campestris pv phaseolin* (Honma, 1956). Anderson *et al*. (1996) suggest congruity backcrossing between *P. vulgaris* and *P. acutifolius* as a way to maintain exotic germplasm with immediate useful forms. *Phaseolus coccineus* has been more commonly used in wide crosses with *P. vulgaris* especially for traits such as cold temperature tolerance and resistance to root rot and bean yellow mosaic virus, and with some interspecific hybridisation being studied (Mok *et al*., 1978). Thomas *et al*. (1983) observed that fruit abortion

occurs in a reproductive unit four days after anthesis, and can be attributed to nutrient diversion to non-aborting axillary and basal fruits four days post anthesis.

2.1.5 The cropping system

The bean production systems used in common bean production vary from location to location depending on the levels of production inputs. In Africa bean production falls within the five production zones of Eastern, Southern, Central, Western and Northern that are a result of interplay of many factors including climate, soil type, and a range of socio-economic and biological factors (Wortmann *et al*., 1998). As a result of these diverse production Agro-ecologies in Africa, common beans are normally grown as a sole crop, maize intercrop, banana intercrop, root or tuber crop intercrop (cassava and sweet potato), sorghum and millet intercrop, and others (Wortmann *et al*., 1998) although maize intercrop dominates in all the beans producing areas. Woolley *et al*. (1991) described 5 broad classes of common bean cropping system for tropical and subtropical conditions as intercropping of beans with other cereals, or with bananas, cassava, coffee, or sugarcane. Woolley *et al*. (1991) further noted that, these intercrops in addition to their domestic importance at household level of these small-scale farmers, they function to provide support to the climbing types of beans. Woolley *et al*. (1993) noted and outlined key critical stages for weeding of beans if yield loss is to be avoided. The beans are grown between 400 to 3000 metres above sea level in major producing areas (Beebe *et al*., 2012), and the production systems are similar to those in Latin America. In North America, Europe and in limited areas of other production regions, much of the bean production is highly commercialized (Graham and Ranalli, 1997) where the beans are grown on levelled fields with mechanization, fertilizer and pesticide inputs, and sometimes with irrigation. In these areas plants of growth Habit I or II predominate since they are the best suited to intensive cultivation, including semi-mechanized harvest (Beebe *et al*., 2012). In Brazil, which is the largest single bean producer in the world currently, bean is grown on the drought-prone sandy soils of the northeast and acid infertile soils of the Cerrados (Thung and Rao, 1999; Ishitani *et al*., 2004), a fact that demonstrates the wide environmental adaptations for the common bean, and a number of efforts being put in place to release drought tolerant bean varieties.

In tropical and subtropical conditions, the production of beans is dominated by women who are all small scale farmers (Wortmann *et al*., 1998; Assefa *et al*., 2005). In Zambia, a survey by Chalwe (2011) reported that bean production is dominated by small scale farmers of less than 0.5 ha (81.5 percent) and those with land holdings of greater than 1 ha constitute only 5.75 percent of

the bean growers. This same survey also reported that 91.76 percent of farmers do not practice any mechanisation at all. The small land holding of less than 1 ha, makes farmers' choices to be restricted on those varieties that can have high yields, so that they can get a good harvest from their production (Assefa *et al*., 2005). In addition to the size of the land, family status, other factors such as gender and farm business orientation affect the choices of what varieties of beans are to be grown. Male farmers are interested to grow varieties that can fetch high prices in the market after harvest while female farmers are interested in varieties with good culinary characteristics; low income farmers prefer early maturing varieties compared to middle to high income farmers; and commercial farmers prefer to grow pure varieties compared to small scale farmers that go for seed admixture (Wortmann *et al*., 1998; Assefa *et al*., 2005; Asfaw *et al*., 2012). Irrespective of the varieties chosen, gender, size of land holdings, amongst other factors affect bean production. Common beans are grown twice in most countries in Africa with some little variations between the planting months amongst countries (Wortmann *et al*., 1998).

2.2 Limitations to yield

Bean production worldwide is severely constrained by a number of factors (Graham and Ranelli, 1997; Wortmann *et al*., 1998; Ojwang *et al*., 2011). Beebe and Pastor-Corrales (1991) categorised these constraints as biological (diseases, pests and weeds), edaphic (poor soil fertility, soil nutrient toxicity – Aluminium), and climatic (drought, high temperature and floods). It is these constraints that this section seeks to explore below:

2.2.1 Common bean diseases

Beebe and Pastor-Corrales (1991) noted that more bean pathogens and virulent isolates of these pathogens are associated with beans in Latin America and Africa than temperate bean growing regions of North America and Europe. Graham and Ranalli (1997) associated this trend to the difference in the farming practices between the subsistence farmers in Africa and Latin America to their counterparts in North America and Europe. Specifically, Graham and Ranalli (1997) noted that the warm and humid environments in the tropics and subtropics favour pathogen development, while the 2-3 cropping cycles per year in some of these regions provide a continuity of inoculum build up. Moreover, small land-holdings limit the possibilities of crop rotations, and the scarcity and cost of disease-free seeds account for the difference in disease development in tropical and subtropical regions compared to temperate regions.

Pathogens of beans with high economic effects and wide distribution in Africa and Latin America include anthracnose *(Colletotrichum lindemuthianum),* rust *(Uromyces appendiculatus var appendiculatus),* common bacterial blight *(Xanthomonas campestris* pv *phaseoli),* bean common mosaic virus (BCMV), bean golden mosaic virus (BGMV), and angular leaf spot *(Phaeoisariopsis griseola)* (Beebe and Pastor-Corrales, 1991; Graham and Ranalli, 1997; Wortmann *et al*., 1998; Muimui *et al*., 2011; Muthomi *et al*., 2011). Other diseases such as halo blight (*Pseudomonas syringae* pv. *phaseolicola*), Charcoal rot (*Macrophomina phaseolina*), Fusarium wilt (*Fusarium oxysporum (F. oxysporum*)), white mold (*Sclerotinia sclerotiorum*), and Ascochyta blight (*Phoma exigua* var. *diversispora* and/or *Ascochyta phaseolorum*) were recognised by Beebe and Pastor-Corrales (1991), as they cause significant crop losses, but tend to be confined to specific environments. A third group of diseases, although widespread, tends not to cause heavy losses, these include web blight (*Rhizoctonia solani*) and Fusarium root rot (*Fusarium solani* f. sp. Hansen) and Southern blight (*Athelia rolfsii*)). Many other diseases are recorded, but occur either sporadically or locally (Allen, 1995). Researchers all over the world assess the economic significance and the amount of the disease that is present by measuring incidence and severity. A disease incidence refers to the proportion of plants in a community that is infected by the particular disease in question, whereas disease severity is the proportion of each infected plant area that is affected (Seem, 1984; Campbell and Neher, 1994). Therefore, disease incidence is done by counting, whereas disease severity is done through scoring on an agreed scale, normally from 1-5, with 1 being very minor/or no damage, and 5 being severely damaged. Campbell and Neher, 1994) noted that for proper and clear disease quantification and an epidemic assessment, there is need for the identification of the needs and goals for disease assessment; consideration of the types of symptoms and signs that may be assessed along with the challenges associated with such assessments; and analysis of the methods and techniques available to quantify root diseases.

2.2.2 Common bean pests

Several insect pests and other invertebrates were evaluated for their importance as constraints to bean production, and have been shown to inflict major damage on beans in both Latin America, Asia and Africa including aphids (chiefly *Aphis fabae*); pod borers (*Helicoverpa spp.* and *Marucatestulalis*); bean stem maggot (*Ophiomyia spp*.); foliage beetles (*Ootheca spp*.); bruchids, including *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say); and thrips (*Megalurothrips sjostedti*) (Kornegay and Cardona, 1991; Graham and Ranalli, 1997; Wortmann *et al*., 1998). These pests have been broadly classified as field and storage pests, and have

varying levels of economic damage depending on the country (Abate and Ampofo, 1996; Abate *et al*., 2000; Schmale *et al*., 2002).

Among the many insects recorded on beans in Africa and Asia, the bean fly complex (bean stem maggots) and the bruchids are the most important ones with very high levels of economic losses (Karel and Autrique, 1989; Abate and Ampofo, 1996; Ojwang *et al*., 2011). Other important insect species in Africa are the black bean aphid, the foliar beetle, the legume pod borer, thrips, and bruchids (Kornegay and Cardona, 1991; Graham and Ranalli, 1997; Wortmann *et al*., 1998). Some of these pests are locally important in Africa, including whitefly (*Bemisia tabaci*) in northern Sudan; *Apoderus humeralis* ('le cigar', a bean leafroller) and Painted lady butterfly *(Pyrameis cardui)* in Madagascar (Rabary 1993, cited in Wortmann *et al*., 1998); and the *Meloids* (pollen and blister beetles, often referred to as 'CMR beetles') in Lesotho, Swaziland, and South Africa.

In Latin America, leafhoppers (*Cicadellids*), leaf beetles (*chrysomelids*), cutworms (*Agrotis spp.*), leaf-feeding caterpillars (*Operophtera brumata, Erannis defoliaria, Alsophila aescularia)*, and storage insects (bruchids) and mites *(Mononychellus tanajoa)* are the most widely distributed bean pests (Kornegay and Cardona, 1991), The bean pod weevil (*Acanthoscelides obtectus*), whiteflies (*Bemisia tabaci*), and, to a lesser extent, the Mexican bean beetle and slugs (*Deroceras reticulatum*) are important in Mexico and parts of Central America (Schoonhoven and Cardona, 1980; Cardona, 1989). In the United States, the Mexican bean beetle (*Callosobruchus maculatus*), the seed corn maggot (*Hylemya cilicrura*), and the two-spotted spider mite (*Tetranychus urticae*) as the main pests (Kornegay and Cardona, 1991). Storage insects, particularly bruchids, force farmers to sell their grain shortly after harvest, and has a significant effect on price fluctuations in the developing country bean market (Graham and Ranalli, 1997), although Baier and Webster (1992) showed that bruchids can be effectively managed using black paper in Colombia. There is less concern under field conditions in Europe and the USA, although leafhopper damage can be significant (Lindgren and Coyne, 1995).

2.2.3 Comparing climatic factor limitations in major beans producing areas

Among the climatic factors as a constraint to bean production, photoperiod response, high temperature, water deficit and air pollution have been shown to cause significant economic losses to areas where common beans are grown (Graham and Ranalli, 1997; Wortmann *et al*., 1998). White *et al*. (1992) showed that photoperiod response affects adaptation of beans to higher

latitudes, with higher sensitivity in genotypes of Andean origin than in genotypes of Mesoamerican origin. Similarly, White and Laing (1989) had also shown that germplasm accessions from higher latitudes were predominantly day neutral, while those from lower latitudes demonstrated responses dependent upon apparent regional differences in altitude of adaptation and in seed-type classes.

In Eastern and Southern Africa, optimal bean production occurs at a mean temperature of 15-23^{o}C (Wortmann *et al*., 1998). This is because bean reproductive development is sensitive to temperature variations (Graham and Ranalli, 1997) as pollen/stigma interaction, pollen germination, pollen tube growth, and fertilization are all negatively affected by high temperature (Konsens, Ofir, and Kigel, 1991; Gross and Kigel, 1994; Porch and Jahn, 2001) with the lowest pod set observed in plants exposed to high temperature 1-6 days prior to the onset of flowering. Despite this challenge of high temperature, Wortmann *et al*. (1998) reported that beans are being produced at relatively high temperatures in eastern Transvaal in South Africa; South-western Sudan; the Phalombe Plains in Malawi; and Bas-Zaire, Eastern Kasai, and Western Kivu in Democratic Republic of Congo (DRC). This demonstrates a lot of efforts that have been put in place in breeding for heat tolerance in common beans by the different common bean improvement centres as reported by Beebe *et al*. (2011).

About 60% of bean production in the developing world (Africa, Brazil and Central America) occurs under conditions of significant drought stress, where the growing season is short and the rainfall is unreliable. Acosta-Gallegos and Adams (1991) documented the plants traits and characteristics under conditions of water deficit so that farmers and breeders in this kind of environment can take advantage of such characteristics. Nunez-Barrios (1991) showed that beans are particularly susceptible to drought during flowering, with significant flower and pod abortion occurring when water shortage occurs at this time. Water deficit hurried flowering and seed fill but delayed leaf appearance. A rapid root expansion was noted at the beginning of the water deficit period and was followed by both root death and compensatory growth in deeper soil layers. This problem of water deficit is particularly common in developing countries in Africa and Latin America. In Europe and USA, water management is a critical factor of bean production with different irrigation regimes designed to replace 30 – 150 percent of the evapotranspiration losses (Barbieri and de Pascale, 1992), but major progress can also be achieved through genetic improvement (White *et al*., 1994; Singh, 2001). Lizana *et al*., (2006) noted common bean

genotypes response differentially to drought, and this can be exploited by breeders to enhance bean production.

Last but not least, among the climatic factors, air pollution in major cities and around industrial complexes is a serious threat to bean production in urban areas. Ozone and sulphur dioxide are the principal pollutants. Bender *et al*. (1990) exposed plants of common bean grown in open topped chambers to doses of ozone ranging from 24-109 parts per billion (ppb). In their study, results indicated that, exposure to ozone did not impair vegetative growth, but did reduce foliar chlorophyll concentration. Concentrations below 70 ppb had little effect on yield, but at 80 ppb yield components were reduced 12-20%.

2.2.4 Comparing edaphic factor limitations in major beans producing areas

Soil constraints are probably the biggest single cause of a persistent yield gap between potential and realized productivity, particularly in developing countries in the tropics (Wortmann *et al*., 1998) and they include soil pH, soil nutrient deficiency, soil salinity, and soil nutrient toxicity mainly with Aluminium (Al) and Manganese (Mn) (Beebe *et al*., 2012). The work of Sanchez and Cochrane (1980) and a review by Graham and Ranalli (1997) reported that soil nutrient depletion is common in most parts of Latin America (Brazil, Columbia, Ecuador, Mexico), and that these soils are deficient in nitrogen , phosphorous , potassium, sulphur, zinc, calcium, and magnesium. The soil nutrients constraints to bean production in East and Southern Africa appear very similar with additional challenges of aluminium and manganese toxicities that has been reported to cause losses of about 100 to 200 kilogram per hectares depending on the soil pH (Semoka *et al*., 1990; Wortmann *et al*., 1998). In order to address these nutrient deficiencies, common bean breeders are exploiting the natural variations in the rate of nitrogen fixations during the symbiotic associations between the bacteria and the plants in the root nodules to provide immediate and dramatic enhancement of biological nitrogen fixation (Graham, 1981; Bliss and Hardarson, 1993; Giller and Cadisch, 1995).

Gama *et al*. (2007) studied the effect of salinity stress on five cultivars of common bean on a sand/peat medium with different salinity levels (0, 50 and 100 mM NaCl) applied 3 weeks after germination for duration of 10 days and reported that, salinity did not affect the biomass yield and relative growth rate only, but also affected other morphological parameters such as plant height, number of leaves, root length and shoot/root weight ratio. They reported further that, photosynthesis, transpiration rate and stomatal conductance were adversely affected in all

cultivars as well as leaf osmotic potential and leaf turgor that varied significantly among cultivars and salt levels. Delgado *et al*. (1994) investigated the effects of salinity on growth, nodulation, acetylene reduction activity (ARA), nodule leghemoglobin (Lb) content and respiratory capacity of bacteroids from pea (*Pisum satiuum cv*. Lincoln), faba-bean (*Viciufabu L. var*. *minor cv*. Alborea), bean (*Phaseolus eulgaris cv*. Contender) and soy bean (*Glycine max L. var*. Williams). Saline stress was also responsible for a decrease in nodulation, and this effect was more pronounced in pea and bean nodules than in soybean and faba-bean nodules. The higher sensitivity of common bean to salinity was reported by Serraj *et al*. (1998) as being associated with a higher accumulation of sodium (Na) and chlorine (Cl) in the nodules and only a small difference between salt-treated and control plants of common bean in their responses. A review by Farooq *et al*. (2017) highlighted that salt stress reduces seed germination by inhibiting water uptake, and reduces growth, mineral uptake, and yields due to ion toxicity and reduced photosynthesis. The review suggested that, seed priming, nutrient management, application of arbuscular mycorrhizal fungi, and integrated breeding and crop management strategies can be used to improve and or manage salt tolerant in common bean.

Soil acidity (low pH of below 4.5) has been shown to affect the growth, development, and ultimately the yields of common bean. Buerkert *et al*. (1990) showed that application of lime to raise soil pH resulted into yield increase of 76 to 313 percent through maintaining a higher plant density at maturity by 23%, 40% greater shoot, 18% greater root dry weight, and improved nodules weight per plant by 110%. Fageria (2008) explained that yield and other parameters' improvement in common bean can be attributed to improving soil pH, base saturation of calcium, magnesium, and potassium and reducing aluminium saturation, and that the optimum values for top soil were pH of 6.5-6.7, base saturation 67% of which Ca is 48%, and Mg is 19%. Hence, from these two authors, it can be seen that, application of lime is an important component in improving acidic soil for common bean production.

In summary, the constraints discussed above pose yield losses at different levels, and their effects are being realised by different countries differently. Beebe *et al*. (2012) observed that a number of interventions are being put in place at different stages of the value chain to mitigate the effects of these constraints to common bean including improving host plant resistance to biotic stress factors, improving nutrient acquisition and use efficiency, improving agronomic management, and reducing carbon footprints through reduced transport and cooking time. Centre for International Tropical Agriculture (CIAT) and Pan Africa Beans Research Alliance (PABRA),

together with the National Agricultural Research Institutes (NARIs) are spearheading the implementation of these interventions, with the exploitation of host plant resistance through breeding being top of the agenda.

2.3 The breeding of *Phaseolus vulgaris*

2.3.1 Common bean breeding objectives

Common bean breeding objectives have been aligned to addressed the constraints affecting common bean production and marketing in the major bean producing areas and include the following: breeding for seed yield and early maturity (Singh, 1991), breeding for adaptation to photoperiod and temperature variations (Masaya and White, 1991), breeding for adaptation to drought (Porch *et al*., 2009; Beebe *et al*., 2011; Porch *et al*., 2013), breeding for disease resistance (Beebe and Pastor-Corrales, 1991), breeding for insect resistance (Kornegay and Cardona, 1991), breeding for food quality factors (culinary quality, nutritional quality) (Shellie-Dessert and Bliss, 1991). In undertaking these breeding objectives, both conventional (classical breeding) and molecular approaches through genetic engineering are being employed (Ishitani *et al*., 2004; Miklas *et al*., 2006a; Beaver *et al*., 2009). Sometimes the use of conventional method is accompanied with the use of molecular techniques such as the use of marker assisted selection to reduce the time period needed to develop a variety (Geffroy *et al*., 1998; Miklas *et al*., 2002; O'Boyle *et al*., 2007). For some time now, it has been observed that the breeders' objectives may be different from the end users' objectives, leading to the development of a gap when it comes to the dissemination of new varieties. To bridge this gap, participatory plant breeding is being implemented by major NARIs in developing countries, and this has increased the rate of adoption for newly released bean varieties (Assefa *et al*., 2005; Asfaw *et al*., 2012). Therefore, a combination of methods and approaches coupled with improved technology, tremendous efforts has been realised in addressing the constraints to common bean production, through the use of both wild relatives or closely related species and landraces of common beans as discussed in the next section.

2.3.2 Use of wild relatives and closely related species in common bean in breeding

The use and understanding of wild crop relatives (WCRs) and closely related species in common been breeding has been utilised adequately (Gentry, 1969; Berglund-Brücher and Brücher, 1976). Further, the understanding that common bean has sister species in the genus is even more recent, therefore, currently the genus *phaseoli* could have six taxa as wild species to date: *P. albescens P. coccineus P. costaricensis P. dumosus P. persistentus* and *P. vulgaris* (Schmit *et al*., 1993;

Delgado-Salinas *et al*., 1999; Delgado-Salinas *et al*., 2006) and the use of wild relatives has been centred on the 5 members of this genus. Porch *et al*. (2013) observed that the greatest difficulties preventing the application of CWRs as a routine tool for crop improvement are lack of knowledge about the genetics of traits of interest in undomesticated and wild germplasm, and the uncertainty about the behaviour of the trait in the genetic background of domesticated bean germplasm. They further proposed that, approaches to uncover CWR genetic variation would include a high-resolution genotyping (via sequencing) and creating populations between CWRs and adapted germplasm to be able to test CWR chromosomal segments for effects on target traits.

Based on the above proposal, specific efforts have been made in the use of these WCRs and promising results are coming up. For instance, seed protein arcelin, which confers moderate levels of resistance to bruchids (*Acanthoscelides obtectus* and *Zabotes subfasciatus*), was originally identified in wild bean accessions from Mexico (Keneni *et al*., 2011). Additionally, Beaver *et al.* (2012) released a web blight and common bacterial blight resistant black bean germplasm line PR0650-31 that was derived from the cross BAT 93/PI 417662//VAX 6 with PI 417662 being a wild type bean germplasm that was collected in Jalisco, Mexico. An attempt to use closely related species has been undertaken on scarlet runner bean (*Phaseolus coccineus* L.) and tepary bean (*Phaseolus acutifolius* A. Gray) as discussed here under. It's also worth mentioning here that the use of either wild relatives or closely related species, has had less progress for quantitatively inherited traits such as tolerance to abiotic stress or seed yield, and therefore more effort is needed to address these components (Blair *et al*., 2006b).

Hybridization between the common bean and the scarlet runner bean which represents a secondary gene pool, has also been met with difficulties due to differences in photoperiod sensitivity, flowering pattern and other factors (Blair *et al*., 2006b). Specifically, Ferwerda and Bassett (2000) identified three incompatibility barriers in crosses between common beans and scarlet runner beans; blocked cotyledon lethal, crinkle leaf dwarf and dwarf lethal that are controlled by complementary dominant gene action. Their study further showed that, the black bean line 5–593 and the snap bean cultivar 'Regalfin' could serve as useful bridging lines to transfer desirable traits from scarlet runner beans to common beans. However, this should involve the use of Congruity backcrossing (CBC) method as described by Singh *et al*. (2009). Based on this approach, several resistance genes have been transferred from scarlet runner bean to common bean, including bean golden yellow mosaic virus (BGYMV) and white mold (Singh *et al.*, 2009), common bacterial blight resistant bean (Miklas *et al*., 1999), aluminium tolerance of

common bean (Butare *et al*., 2011), resistance to *Ascochyta* blight in Colombia (Schmit and Baudoin, 1992), both recessive gene and dominant gene (Osorno *et al*., 2007) that control resistance to leaf chlorosis and BGYMN respectively, and white mold resistance was also shown to be controlled by a single dominant gene (Schwartz *et al*., 2006).

Conversely, hybridization between common bean and tepary bean has been more challenging, and requires the use of embryo rescue to secure viable embryos and breeding methods (Thomas *et al*., 1983; Mejía-Jiménez *et al*., 1994). This is because tepary bean is a more distant relative of common bean that represents a tertiary gene pool, and have their origin in warmer and more arid environments than common bean, as tepary bean landraces have shown to be superior heat (Nabhan, 1979) and drought tolerance (Markhart, 1985; Federici *et al*., 1990; Porch *et al*., 2009). Tepary bean therefore, represents a valuable source of traits for heat and drought tolerance, insect and disease resistance, although early attempts to transfer many of these traits have had limited success due to their genetic complexity and resultant F1 sterility (Thomas *et al*., 1983). The use of recurrent backcrossing (i.e., repeated backcrossing to one of the parents) to both parents independently and for congruity backcrossing (i.e., backcrossing alternately to both parents) methods have been employed to produce fertile intermediate hybrids between *P. acutifolius* and *P. vulgaris* (Haghighi and Ascher, 1988; Mejía-Jiménez *et al*., 1994; Urrea and Singh, 1995). Congruity backcrossing (CBC) particularly has been adopted by the bean breeding programs as an effective means to force introgression and eliminate barriers such as embryo abortion and hybrid sterility between distant species (Mejía-Jiménez *et al*., 1994). Using CBC method, several traits have been transferred from tepary bean to common bean including common bacterial blight (CBB) resistance (Scott and Michaels, 1992; Singh and Munoz, 1999; Osorno *et al*., 2013), BGYMN resistance (Miklas and Santiago, 1996), BCMNV resistance and bruchids (*Acanthoscellides obtectus*) resistance (Kusolwa and Myers, 2011), ashy stem blight and Fusarium wilt (*F. oxysporum*) resistance (Miklas *et al*., 1998), and bean rust resistance (Pastor-Corrales *et al*., 2011). Further studies have been undertaken to select interspecific lines with more promiscuous nodulation and/or improved biological nitrogen fixation (Brink and Belay, 2006) due to difference in tepary bean (*Bradyrhizobium* spp) and common bean (*Rhizobium* spp) for nodulation.

2.4 Genetic diversity among *Phaseolus vulgaris*

Common bean domestication took place from the two highly differentiated geographic areas, and the use of wild or close relatives in breeding programmes have resulted in a very high level of

diversity both morphologically and genetically for the crop. In order to understand this diversity and the geographical differentiation (Figure 2.2) in common beans, scientists have used a number of approaches. For instance, seed size and growth habit (Evans, 1976; Lima *et al*., 2012), environmental adaptation (Kelly *et al*., 1987), infertility barriers (Singh and Gutiérrez, 1984; Gepts and Bliss, 1986), isozyme diversity (Koenig and Gepts, 1989; Paredes and Gepts, 1995; Santalla *et al*., 2002), phaseolin types (Gepts and Bliss, 1986; Gepts *et al*., 1988; Kami *et al*., 1995; Islam *et al*., 2002; Kwak and Gepts, 2009), nuclear RFLP (Restriction fragment length polymorphism) diversity (Becerra and Gepts, 1994; Becerra *et al*., 2010), RAPD (Random amplified polymorphic DNA) diversity (Vera *et al*., 1999; Palomino *et al*., 2005; Szilagyi *et al*., 2011), AFLP (Amplified fragment length polymorphism) diversity (Tohme *et al*., 1996), and SSR (Simple sequence repeat) diversity (Yu *et al*., 2000; Gaitan-Solis *et al*., 2002; Grisi *et al*., 2007; Blair *et al*., 2003; 2006a; 2007; 2009a, b; 2010; 2012; Angioi *et al*., 2009a and 2009b; Becerra *et al*., 2010; Burle *et al*., 2010; Wang *et al*., 2012; Raggi *et al*., 2013; Okii *et al*., 2014b; Felix *et al*., 2014). Some studies have use a combination of approaches to give a better insight into the genetic diversity and gene pool differentiation (Chiorato *et al*., 2006; Cabral *et al*., 2010; Ligaretto and Martinez, 2014). The ability to combine two or more methods indicate that some methods are better than others, and hence most of the studies have relied on a combination of agro-morphological traits and molecular approaches.

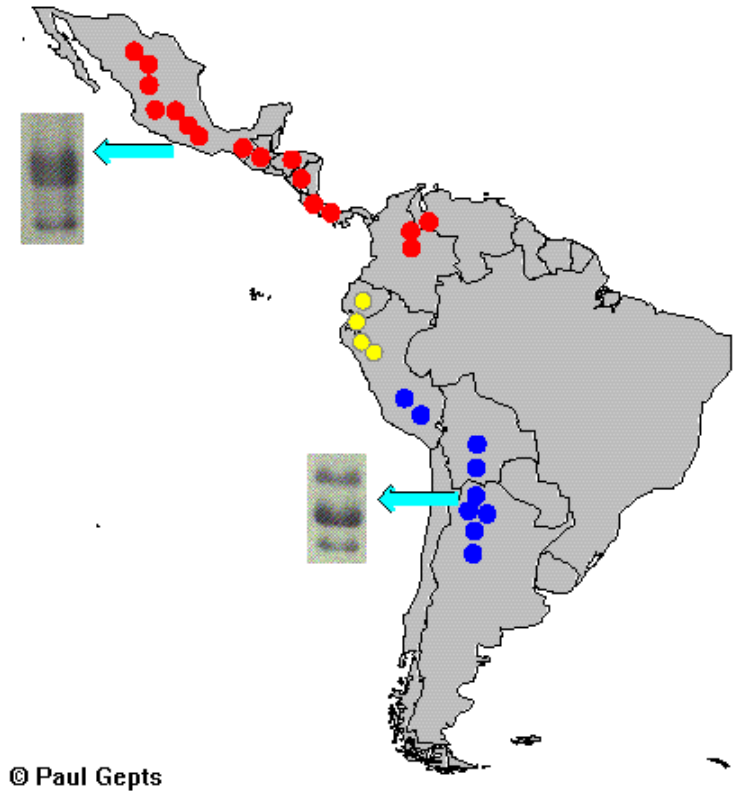


Figure 2.2 Differentiation of the common bean into two gene pools in Mexico and the Andean areas. Red Represents Mesoamerican gene pool, Blue represents the Andean gene pool and the Yellow represents hybridization potentials between the two gene pools. Adapted from Gepts *et al*., 1986.

2.4.1 Morphological diversity

Other than the molecular and biochemical approaches to study genetic diversity in common bean discussed in the later sections, the first approach to understand diversity of a crop germplasm is to use morphological characters. These Agro-morphological descriptors range from hypocotyl colour, plant growth habit, seed colour, seed shape, flower colour, stem colour, pod colour, pod break position, among many others; and can be grouped as qualitative and quantitative traits during the study process (Hornakova *et al*., 2003; Hegay *et al*., 2013; Awan *et al*., 2014). In addition to these qualitative and quantitative traits, Ligarreto and Martinez (2014) introduced the concept of physiological characters where traits such as total leaf area, root, stem, pod and seed dry weight, chlorophyll a and b, as well as their proportions, proportions of leaf, stem, flowers, pods, seeds and root dry weights, and others were measured.

These studies based on agro-morphological descriptors in common beans have provided very useful information on diversity among common beans for certain locations, and are used to

identify useful agro-morphological traits for common bean improvement. Awan *et al*. (2014) used morphological characters to study the diversity of common beans in Pakistan, and their study identified duration to flowering, number of pods, number of seeds and grain yield to have a strong positive correlations and thus useful traits for bean improvement in Pakistan. Hegay *et al.* (2013) showed that the common bean from Kyrgyzstan clearly were distinguished between the Andean and Mesoamerica using the agro-morphological descriptors, and they further showed that the Andean gene pool were less diverse compared to the Mesoamerican gene pool in this area. Chiorato *et al*. (2006) used a combination of agro-morphological and molecular techniques to identify duplicates in common beans in Brazil. Their study further identified pod and seed traits as very varying and appropriate to use as morphological descriptors to study genetic diversity in common bean. Lima *et al*. (2012) used morphological descriptors in common beans in Brazil to identify high yielding varieties that were recommended to be used as parental breeding lines, and they showed that 10-20 descriptors are appropriate to study the characterisation of genetic diversity in common bean.

In the Eastern and Southern African context, Okii *et al*. (2014b) showed that there is moderate genetic diversity of the common bean germplasm from Uganda. Their agro-morphological descriptors were able to classify these germplasm into three clusters, a situation that was similar to their finding (Okii *et al*., 2014b) using molecular approaches. These studies in Uganda reported by Okii *et al*. (2014a and 2014b) showed clearly how agro-morphological characterisation is important in complementing the molecular characterisation. Besides, it's the agro-morphological characters that farmers and breeders normally depended on when it comes to participatory plant breeding during participatory variety selection (PVS). Aspect of seed size and colour affect the marketability of seeds in urban and rural locations differently, as urban setting such as towns and cities require pure seeds their counterparts in the rural markets accept seeds with varying levels of seed mixture (Assefa *et al*., 2005; Asfaw *et al*., 2012). Therefore, it is important to combine all the approaches when characterising germplasm from a particular region/country.

2.4.2 Molecular Diversity

Molecular diversity in crop species including common bean has been studied using different marker systems. For common beans, the following marker systems have been found useful and informative in characterising and assessing the genetic diversity and population differentiation. However, as technology advances, some of these marker systems are no longer in use but they

have provided very useful information as far as the genetic diversity of common bean is concerned.

2.4.2.1 Phaseolin seed proteins and Isozymes

Seed protein and isozyme variability have been used extensively in many crops, including common bean, to detect patterns and levels of genetic diversity within a species (Ladizinsky 1983; Loveless and Hamrick 1984). Phaseolin is the major seed storage protein of common bean (Orsbone 1924) that are encoded by a small gene family of 6-10 that are tightly linked sequences on chromosome 7 (Talbot *et al*., 1984; Nodari *et al*., 1993). These proteins have further been used in the studies of genetic variation at the phaseolin locus (*Phs*) to identify patterns of multiple domestication and genetic diversity during domestication in the common bean (Gepts, 1990 and 1993). These genetic studies have shown that the genes coding for different polypeptides of each phaseolin pattern are tightly linked and inherited as a single Mendelian unit with the alleles being codominant (Brown *et al*. 1981a, b and 1982). Phaseolin proteins have been reported to display a number of characteristic banding patterns depending on the accessions when subjected to one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/ PAGE) or two-dimensional isoelectric focusing SDS/PAGE (IEF-SDS/PAGE) and this explains why most studies on them utilise these techniques. Gepts and Bliss 1986 and Gepts *et al*. (1986) had shown the major phaseolins types in common beans are “S”, “C”, “H”, “T” and “CH” on which many study rely upon to assess genetic diversity and population structure (Figure 2.3).

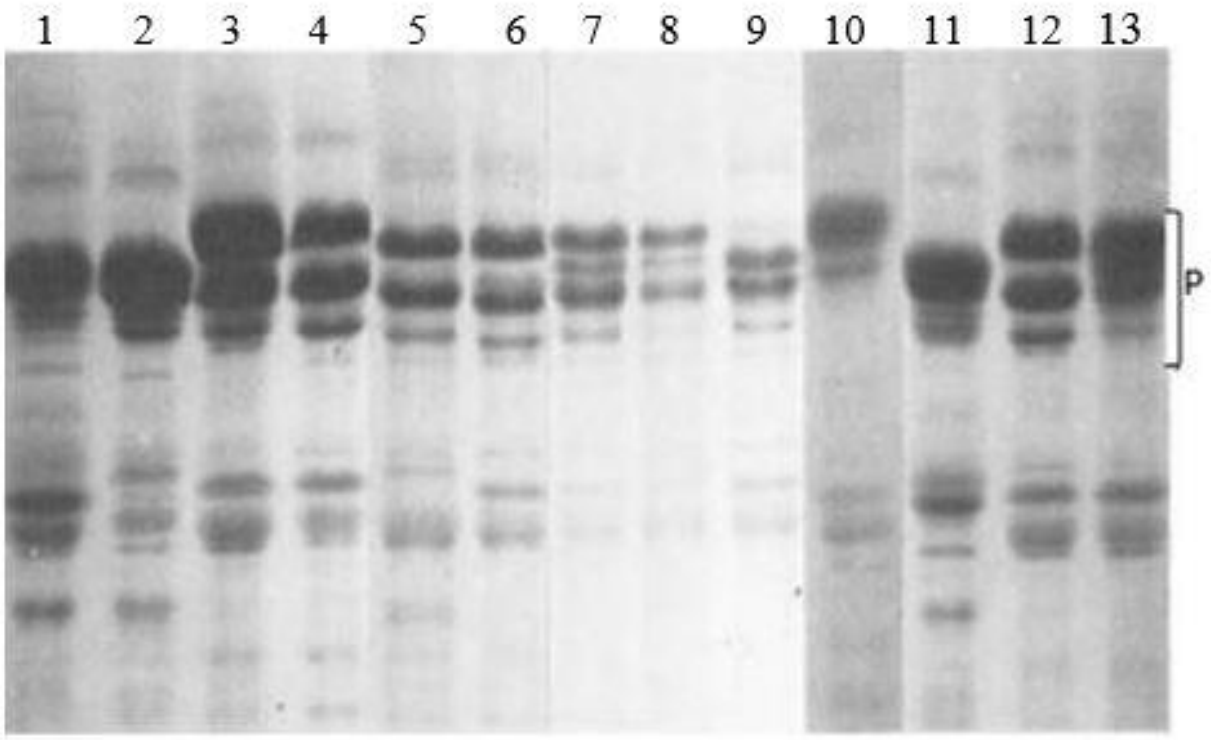


Figure 2.3 One-dimensional SDS/PAGE of Phaseolin types from Landraces: Middle America (1-4) and Andean South America (5-6). 1-2: 'S' phaseolin; 3-4: 'T' phaseolin; 5-6: 'T' phaseolin; 7-8: 'C' phaseolin; 9: 'H' phaseolin10: 'A' pahaseolin and 11-13: S, T and C of cultivars Sanilac, Tendergreen, and Contender, respectively. Modified from Gepts *et al*., 1986.

The two most prevalent phaseolin protein types among cultivars are the "S" and "T" types (Brown *et al*., 1982; Gepts and Bliss, 1986; Gepts *et al*., 1986) although other studies of wild and cultivated common bean genotypes have revealed that: wild genotypes of different geographic origins could be distinguished by their phaseolin type ("S" and "M" in Mesoamerica; "B" and "CH" in Colombia; and "T" in the Southern Andes) as reported in Sullivan and Freytag (1986) and Koenig *et al*. (1990).

Not until recently, a limited number of studies have utilized isozymes to determine genetic diversity and phylogeny relationships in *Phaseolus* and other plant species. Bassiri and Adams (1978a) examined the isozyme variability of esterase, acid phosphatase and peroxidase of 13 species within the genus *Phaseolus,* and found that most species showed unique banding patterns in each isozyme system, with the exception of cultivated *P. Vulgaris,* wild *P. vulgaris* and *P. eoccineus,* which had similar banding patterns. Again, Bassiri and Adams (1978b) examined genetic diversity in 34 cultivars of *P. vulgaris* belonging to 19 commercial classes, and concluded that the isozymes peroxidase and esterase were suitable markers for cultivar identification and for estimating the genetic relationships among cultivars of the same class or among classes. Koenig

et al., (1989) performed allozyme analysis on 83 wild *P. vulgaris* accessions, representing a wide geographical distribution from Mesoamerica to Argentina, to determine levels of genetic diversity and geographic patterns of variability at nine polymorphic isozyme loci, and using 14 enzyme systems. Wild accessions of *P. vulgaris* showed allozyme variation in 8 of the 14 enzyme systems and the 14 enzyme systems showed 22 bands of activity, 10 (45%) of which corresponded to polymorphic loci that were used in genetic distance analyses. Importantly, this study revealed that a number of allozyme that were found only in this specific geographical locations. For example, almost all of the accessions from Mexico, Central America and Colombia showed the 100 allele of *Lap-3*, while accessions from Argentina showed the 103 allele of *Lap-3*. Allele 100 of *Diap-1* was found primarily in accessions from Central America, Argentina and southern Peru. Accessions from Mexico and Colombia had a higher frequency of allele *Diap-1*^{95}. All of the accessions from Argentina had the allele 100 of *Skdh.* Genetic diversity within a common bean collection, comprising 343 accessions from the Iberian Peninsula, was examined using six allozyme markers (Santalla *et al*., 2002). Using isoenzyme analysis, Mandák *et al*. (2005) determined the degree of genotype variability in all four taxa of the genus *Reynoutria* and compared clones of *R. japonica var. japonica* from the Czech Republic with those from Great Britain. The results of their study showed that a rarely occurring tetraploid *variety R. japonica var. compacta* possesses low variability, whereas the octoploid female clone of *R. japonica var. japonica* is genetically uniform in the 93 clones sampled and belongs to the same genotype that is present in the whole Europe.

2.4.2.2 Random amplified polymorphic DNAs (RADP) markers

Haley *et al*., (1994) and Miklas *et al*., (1993) reported that RAPD markers can be used for indirect selection of progeny with pyramided resistance genes. Following their publications, Johnson *et al*., (1995) investigated the usefulness, distribution, and degree of recombination between the RAPDs and the Guatemalan black bean (PI 181996) resistance to the bean rust fungus using an array of Andean and Mesoamerican common bean genotypes. They reported to have found two random amplified polymorphic DNA (RAPD) markers OAC20490 tightly linked (no recombinants) in coupling phase and OAE19890 linked in repulsion phase (at 6.2+2.8 cM) to PI 181996 rust resistance. The RAPD technology is well suited to DNA fingerprinting (dos Santos *et al.,* 1994; Thormann *et al.,* 1994; Garcia *et al*., 2004) although Demeke *et al.,* (1997); and Karp *et al.*, (1997) reported in their studies that RAPD suffers from a certain lack of reproducibility due to mismatch annealing. Zhang *et al*., (2008) investigated the genetic diversity of Flue cured Tobacco (*Nicotiana tabacum L.*) in China using 200 RAPD Markers. They reported

that, from the 200 markers used in RAPD analysis, 63(31.5%) produced the amplification products that were too faint to score or could not be consistently reproduced, and 124 (62%) produced monomorphic banding patterns, and only 13 (6.5%) out of 200 markers were scored. This work by Zheng and the co-authors supports the shortcomings associated with RAPD markers.

2.4.2.3 Amplified fragment length polymorphism (AFLP) Markers

The Amplified fragment length polymorphism (AFLP) technique was regarded as a novel polymerase chain reaction (PCR) based molecular marker assay (Vos *et al*., 1995), which has the capacity to detect a higher number of polymorphic loci in a single assay than RFLPs or RAPDs (Powell *et al*., 1996), which has been used in different crop species. Xu *et al*. (2000) used AFLP markers to characterise the cultigen, wild, weedy, and complex population of Azuki bean (*Vigna angulari*) in Japan. The results of their work indicated that the wild and weedy types were more dispersed than the cultigen on a PCA plot, which is a reflection of their greater genetic variation and the ability of AFLP markers to separate these populations. Maciel *et al*. (2003) studied the genetic variation and relationships among 31 accessions of *P. vulgaris* consisting of landraces and commercial lines from Brazil, together with other commercial lines from Italy, Peru, and USA, plus two representatives of *Vigna unguiculata*, by AFLP analysis. Their results showed that more than 95% of the amplification products showed polymorphism, indicating high variation at the DNA level among these accessions. The Mexican common bean cultivars were analysed using amplified fragment length polymorphism (AFLP) fingerprinting to examine the genetic relationships within and among races, based on the genotyping of 112 bred cultivars developed in Mexico (Rosales-Serna *et al*., 2005), and the results showed that there was broad genetic diversity within bean races, and diversity values between races were similar, confirming the ability of AFLP to resolve genetic differences in some crop species. In yet another separate study, 154 AFLP polymorphic fragments were used to assess the genetic similarity among selected accessions at the South China Tobacco Breeding Research Centre (Liu *et al*., 2009). The results of their study showed that AFLPs seemed to be an effective classification tool for germplasm conservation and breeding, although limited genetic variation was detected within this group of accessions.

2.4.2.4 Single Sequence Repeat (SSR) Markers

SSR markers comprise tandem repeats of short (2-6 base pairs (bp)) DNA sequences that are abundant in the genome, co-dominantly inherited, highly polymorphic and reproducible

(Morgante and Olivieri, 1993; Powell *et al*., 1996). Studies that have used flanking PCR primers have shown that chloroplast SSRs (cpSSRs) are polymorphic among different species and accessions of *Glycine* (Powell *et al*., 1996; Xu *et al*., 2002), Hordeum (Provan *et al*., 1999), Oryza (Ishii and McCouch, 2000), Pinus (Cuenca *et al*., 2003), Solanum (Bryan *et al*., 1999; Sukhotu *et al*., 2006), Vitis (Arroyo-Garcı´a *et al*., 2002), Anthyllis (Nanni *et al*., 2004) and Phaseolus spp. (Sicard *et al*., 2005). This high level of polymorphism that arises from site-specific length variation of the repeat units (Morgante and Olivieri, 1993; Proven *et al*., 2001) make SSRs ideal for studying populations. Comparison of RFLP, RAPD, AFLP and SSR marker systems for germplasm analysis has confirmed the superiority of SSR markers (Powell *et al*., 1996; Rakoczy-Trojanowska and Bolibok, 2004). For instance, Palomino *et al*., (2005) studied genetic diversity in common bean using RAPD markers, where they found out that only 12 of the 49 markers were polymorphic, and Szilagyi *et al*., (2011) also found only 4 RAPD markers polymorphic with the common beans, hence putting SSR markers at an advantage, whereas with SSR markers polumorphism of over 70% have been reported (Asfaw *et al*., 2009; Blair *et al*., 2009a, b, 2010; Okii *et al*., 2014b, Blair and Lorigados, 2016).

Because of their informativeness, SSRs have had various applications in common bean: i) assessment of genetic diversity (Yu *et al*., 2000; Gaitan-Solis *et al*., 2002; Grisi *et al*., 2007; Blair *et al*., 2003; 2006a; 2007; 2009a; 2010; 2012; Becerra *et al*., 2010; Burle *et al*., 2010; Wang *et al*., 2012; Raggi *et al*., 2013; Okii *et al*., 2014b; Felix *et al*., 2014), ii) construction of linkage maps (Yu *et al*., 2000; Blair *et al*., 2003; Pedrosa-Havand *et al*., 2008), iii) mapping of QTL for Anthracnose (Ragagnin *et al*., 2003; Miklas *et al*., 2002), Bean common mosaic virus (Kelly *et al*., 1994;), Bean golden yellow mosaic virus (Miklas and Santiago, 1996; Beaver *et al*., 2012), common bacterial blight (Miklas *et al*., 2003 and 2006b; Mutlu *et al*., 2005), bean rust (Stavely, 1998), and white mold (Miklas, 2007), iv) tracing the origin of common bean (Bellucci *et al*., 2014), v) evaluating interspecific and intraspecific specific diversity between and/or within the genus (Gaitan Solis *et al*., 2002; Blair *et al*., 2010; Felix *et al*., 2014) and vi) fingerprinting genetic diversity in commercial varieties of the common bean from Europe (Me´tais *et al*., 2002; Masi *et al*., 2003) and landraces of beans from Nicaragua (Gomez *et al*., 2004). As a result these molecular markers have contributed immensely to the understanding of common bean evolution and genetics and still offer tremendous scope for the understanding of bean genetics and its improvement as shown in Figure 2.4 a.

Several studies as indicated in the above paragraph using SSRs have determined the genetic diversity of the common bean and its structure in recent years showed that different gene pools have been represented in different proportions in different continents/locations. For instance, in China and Brazil the Mesoamerican gene pool predominates, while in Europe the Andean gene pool predominates, interestingly in Africa both gene pools appear to be present in almost equal proportions overall, but this varies from country to country (Angioi *et al*., 2010 and 2011; Bellucci *et al*., 2014). Analysis of common bean germplasm from Uganda showed that Andean and Mesoamerican gene pools were present in almost equal proportions, 51 versus 49 percent respectively (Okii *et al*., 2014b). The appearance of the two gene pools in equal proportions in African common beans and the admixture planting strategy practice by African farmers, make this region genetically diverse in terms of common bean and hence the study of its germplasm to be of paramount importance to both the breeders and farmers for bean improvement programme.

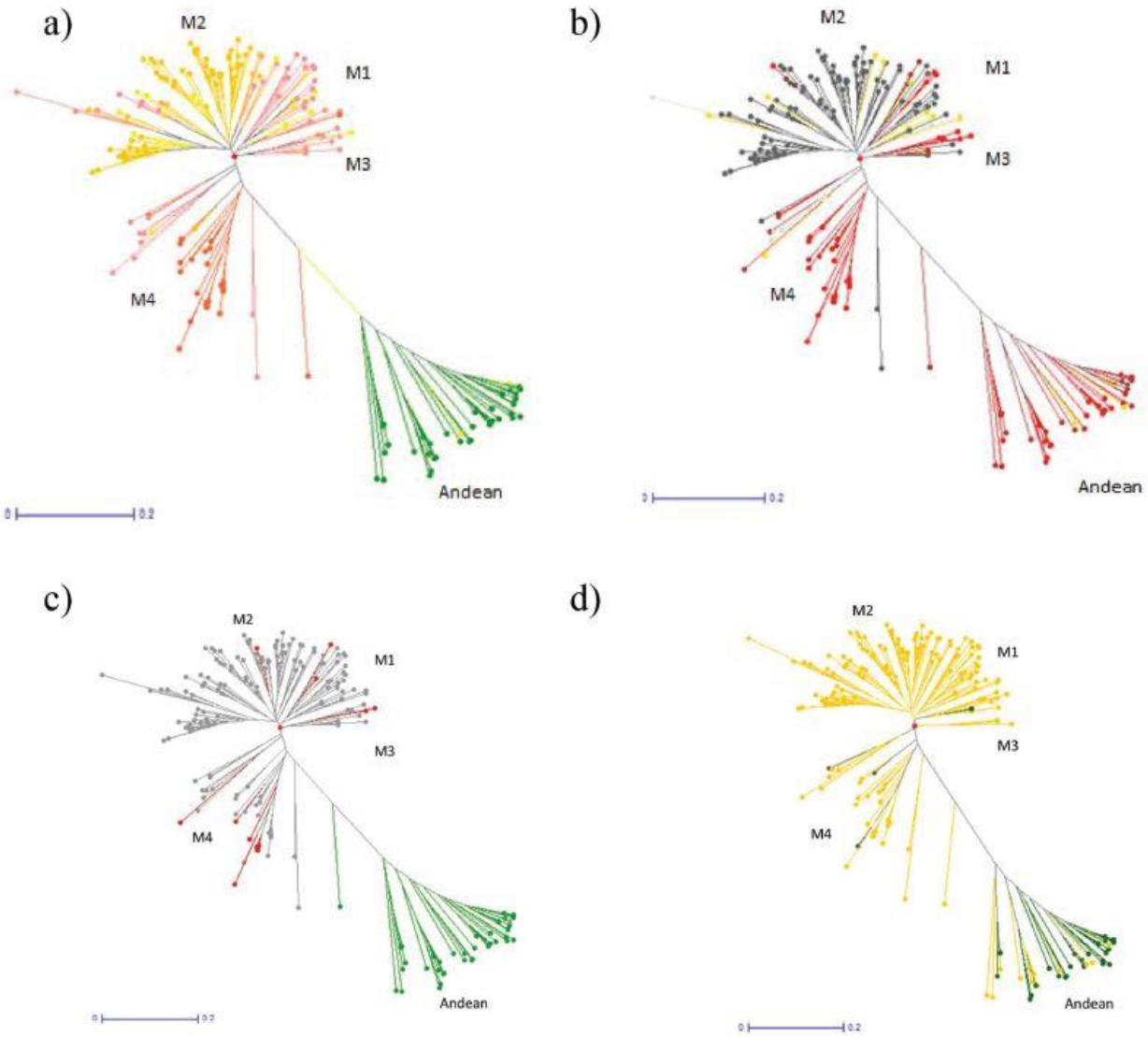


Figure 2.4 Neighbor joining dendrogram for the Cuban common bean genotypes from the Andean and Mesoamerican genepools. Evaluated with: a) 36 microsatellite loci with different line shading or colouring indicating sub-populations at *K* = 5 as M1, M2, and M3 indicating race Mesoamerica subgroups, M4 an introgressed group in the middle, and the Andean group below; b) seed colour, ranging from white (light gray lines), cream (light tan lines), pink (pink lines), red and red mottled (red lines), purple mottled (magenta lines) to black (dark gray lines); c) phaseolin types CH (red lines), S (gray lines), and T (dark green lines); and d) growth habit types determinate (dark green lines) or indeterminate (yellow lines). Adapted from Blair and Lorigados, 2016.

2.4.2.5 EST-SSR Markers

Another technique in the use of molecular markers is the use of microsatellites developed from expressed sequence tags (EST-SSRs) that has been used in Strawberry (*Fragaria ananassa*) (Folta *et al*., 2005), common bean (Kumpatla and Mukhopadhyay, 2005; Hanai *et al*., 2010;

Blair *et al*., 2011), and other plant species (Varshney *et al*., 2002). Kumpatla and Mukhopadhyay (2005) use computational approaches to mine the ever increasing sequences such as expressed sequence tags (ESTs) available in public databases that permit rapid and economical discovery of SSRs from 55 dicotyledonous species, including the common bean. This was after the observation that most of the large scale, multi species in silico mining efforts have focused on monocotyledonous crops (Kantety *et al*., 2002; Thiel *et al*., 2003). Specifically, for common bean, Kumpatla and Mukhopadhyay (2005) found 93 ESTs containing SSR markers, with dinucleotide repeats found to be the most abundant followed by tri- or mono-nucleotide repeats. Blair *et al*., (2011) screened a total of 3,123 EST sequences from leaf and root cDNA libraries and used for direct simple sequence repeat discovery and found 184 microsatellites; the majority containing tri-nucleotide motifs, many of which were GC rich (ACC, AGC and AGG in particular). EST-SSR markers are becoming very commonly used tools as the development of SSR markers using conventional molecular methods is time consuming, laborious, and expensive (Kumpatla and Mukhopadhyay, 2005).

2.4.2.6 Single nucleotide polymorphism (SNP) markers

The development and application of next generation sequencing (NGS) technologies has allowed for the identification of several thousands of SNPs within a single experiment (Stapley *et al*., 2010). This enables single nucleotide polymorphism (SNP) and insertion–deletion (InDel) detection and genotyping to become feasible on a whole genome scale and, as a result, this approach is widely applied to diversity and association studies in plants (Thudi *et al*., 2012; Varshney *et al*., 2014). Nevertheless, the cost of sequencing and genotyping large numbers of individuals is still a big challenge in plants with complex and repetitive genomes (Deschamps and Campbell, 2010). As a result, complexity reduction approaches that couple restriction enzyme (RE) genome digestion with NGS and SNP calling have been developed in the last years for high throughput molecular marker discovery in different organisms (Davey *et al*., 2011). Ariani *et al*. (2016) showed that genotyping by sequencing (GBS) using a reduced representation library approaches is a robust, high-throughput, cost-effective, and simple technique for obtaining thousands of SNP markers from large numbers of individuals using CviAII as restriction enzymes in common bean.

Different authors have provided useful information in relation to the development and use of SNP markers, for instance: i) Hart and Griffiths (2015) found good SNP coverage in common bean using *ApeKI* as the restriction enzyme, but there was uneven density distribution, probably

because *ApeKI* is a methylation-sensitive enzyme; ii) Zou *et al*. (2014) employed a methylation-insensitive enzyme (*HaeIII*) in common bean, but detected a high proportion of the SNPs (approx. 77 %) in repetitive regions; iii) Blair *et al*., (2013b) utilized a 768 feature, Illumina Golden Gate assay for common bean developed from conserved legume gene sequences for the evaluation of parental polymorphisms in a mini core set of common bean accessions and also for the analysis of genetic diversity in the crop; iv) Goretti *et al*., (2014) developed 60 new SNPs markers for KASpar assay genotyping in common bean; v) *Cortés et al*. (2011) reported the development of 94 SNPs and tested them across well-chosen common bean germplasm; vi) Hyten *et al*. (2010) reported 3,487 SNPs using a multitude of reduced representation library; vii) Galeano *et al*. (2009) reported 56 EST-based amplicons designed for SNP containing contigs and presented their primer sequences and melting and annealing temperatures and whether polymorphism by single stranded conformation polymoriphism (SSCP) was detected in the DOR364 × G19833 mapping population; viii) Souza *et al*., (2012) identified 677 SNPs, including 555 single-base changes (295 transitions and 260 transversions) and 122 small nucleotide insertions/deletions (indels), using resequencing of sequence-tagged sites (STSs) developed by PCR primers previously designed to soybean shotgun and bacterial artificial chromosome (BAC) end sequences, and by primers designed to common bean genes and microsatellite flanking regions; and ix) Gaitán-Solís *et al*., (2008) assessed the frequency of SNPs in 47 fragments of common bean DNA, using SBE as the evaluation methodology and conducted a sequence analysis of 10 genotypes of cultivated and wild beans belonging to the Mesoamerican and Andean genetic pools of *P. vulgaris*, amongst others.

Genome wide SNPs discovery by resequencing efforts have been performed in many key legume crops such as tepary bean (Gujaria-Verma *et al*., 2016), Pea *(Pisum sativum)* (Sindhu *et al*., 2014), Chickpea *(Cicer arietinum)* (Hiremath *et al*., 2012), and Faba bean (*Vicia faba)* (Kaur *et al*., 2012 and 2014); and have been applied for different purposes such as genome wide diversity study, association mapping, and genotyping in a selection context (Valdisser *et al*., 2016), as well as capturing broad genetic diversity in genomic assisted breeding for agronomic quality traits in common bean (Rodriguez *et al*., 2016). For common bean in particular, Valdisser *et al*., (2016) using restriction-associated DNA (RAD) sequencing, and Ariani *et al*., (2016) using GBS showed that the two gene pools affect the application of markers. Both authors showed that, SNPs markers reveal more variation in the Mesoamerican beans than the Andean beans. Valdisser *et al*., (2016) further showed that SNPs markers are effective is separating Mesoamerican from Andean beans, but not so in separating individuals within a single gene pool.

This implies that for common beans technologies will keep coming and scientists will continue to use what is available to them, and hence the need to be prepared for the new technologies.

2.4.2.7 Molecular markers from transposable elements

Transposable elements (TEs) are discrete regions of DNA that can move within genomes (Baranek *et al*., 2012), which have been used for phylogenetic analysis of different plants due to the fact that they can change their genomic location, creating genomic diversity. Two classes of TEs are known according to their mode of transposition (Figure 2.5): class I elements transpose through an RNA intermediate (Retrotransposons), while class II elements transpose directly via a DNA intermediate (Casacuberta and Santiago, 2003). Retrotransposons are the most abundant and widely distributed genetic elements in Eukaryotic genomes and show great polymorphism between species, and they play further important roles in plants according to the genome size, structure, evolution, variable copy number, and random distribution (Kumar and Bennetzen, 1999). Some studies conducted using retrotransposon-based markers, such as sequence specific amplified polymorphism (SSAP), inter-primer binding site (iPBS) markers, and inter-retrotransposon amplified polymorphism (IRAP) markers have shown that these markers have a higher discriminatory power than the standard DNA markers such as AFLP, RFLP, and RAPD in genetic diversity analysis (Breto *et al*., 2001; Sensi *et al*., 2003; Labra *et al*., 2004; Kalendar *et al*., 2010 and 2011; Duan *et al*., 2015).

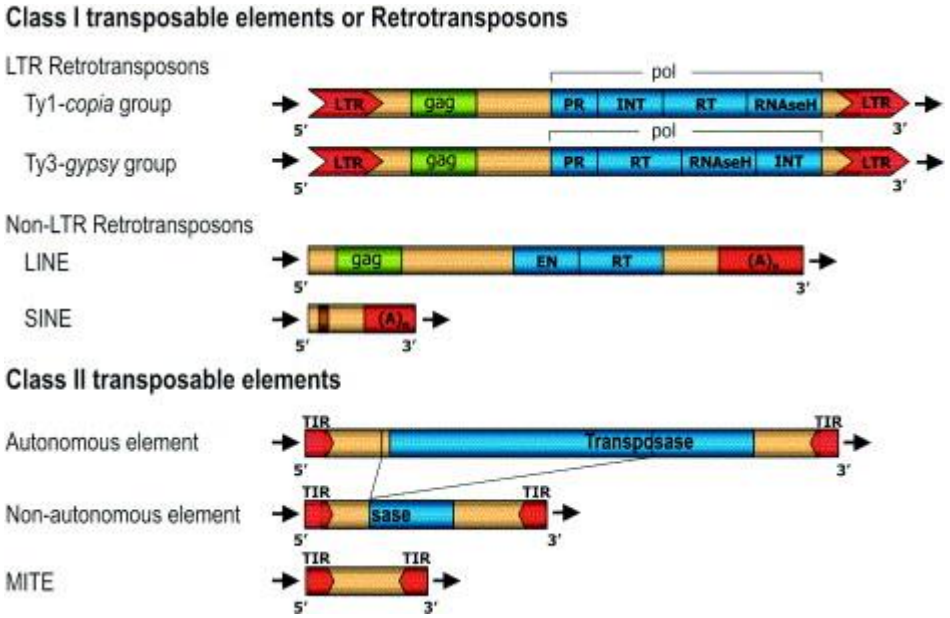


Figure 2.5 Classification of transposable elements and their sub-division within each class. Adapted from Casacuberta and Santiago, 2003.

Kalendar *et al*. (2010) has recently used a relatively new universal retrotransposon-based marker system for DNA fingerprinting, inter primer binding sites (iPBSs), and showed that iPBSs play an important role in the formation of many important traits of plants. iPBS primers are designed to correspond to the conserved parts of primer binding site sequences among different LTR retrotransposon families (Monden *et al*., 2014), and has several advantages compared with other retrotransposon markers, in that, iPBSs can discriminate among genotypes without prior sequence knowledge and are highly reproducible due to their primer length and the high stringency achieved by the annealing temperature (Guo *et al*., 2014a and 2014b). The iPBS marker method has been used successfully for several genetic diversity studies in plants, such as apricot (*Prunus armeniaca*) (Baranek *et al*., 2012), Chickpea (*Cicer* spp) (Andeden *et al*., 2013), and Grape vine (*Vitis vinifera)* (Guo *et al*., 2014a, 2014b), *phaseolus vulgaris* (Nemli *et al*., 2015), amongst others.

In summary, several molecular markers and techniques have been used to study genetic diversity, gene flow, domestications and evolution of many crops species. What remains apparent is that, yet more new methods will keep coming, and that these methods described above, work best for particular crops species than others. For instance, Pejic *et al*., (1998) used a comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs and concluded that all methods could clearly distinguish all 33 inbred lines, although the SSR data provided the highest level of discrimination between any pair of inbreeds; Röder *et al*., (1995) compared restriction fragment length polymorphism (RFLP) and SSR by studying abundance, variability and chromosomal location of microsatellites in wheat, and they found that the microsatellite markers were significantly more variable than the RFLP markers, although it has a very low amplification percentage of 36 percent, which indicates difficulties of using SSRs in Wheat.

The above observations on the superiority of some molecular markers over other and crop species basis have led some studies to use combined methods of either molecular-molecular combinations or molecular-morphological combinations in trying to resolve important questions. For example, Chiorato *et al*., (2006) used agro-morphological and molecular data for the identification of common bean duplicates; Ligarreto, Gustavo, and Martínez (2014) identified the variability of a common bean collection through morphological, physiological, biochemical, and molecular relationships; and Cabral *et al*., (2010) quantified diversity among common bean accessions using the Ward-MLM strategy. As mentioned above, not all approaches are equally

informative with particular crop species, additionally new approaches are coming on stream all the time. Therefore, it merits a brief look at the latest biotechnology approaches in common bean development and improvement in the section below.

2.5 Latest and common biotechnology approaches being used in *Phaseolus vulgaris* improvement

In recent years, there have been more advanced studies directed towards the improvement of the entire value chain of common bean from addressing production constraints, through marketing, and to the final utilisation and consumption the produce. This section therefore presents some of these technologies directed specifically to common beans such as reference genome assembly, genome wide associations and genes discoveries, pathogen detection, and comparative transcriptome analysis as presented below:

Schmutz *et al*. (2014) developed a reference genome for common bean and performed a genome-wide analysis of the dual domestications. They assembled 473 Mb of the 587 Mb genome and genetically anchored 98% of this sequence in 11 chromosome-scale pseudomolecules. Using resequencing of 60 wild individuals and 100 landraces from the genetically differentiated Mesoamerican and Andean gene pools, the team confirmed 2 independent domestications from genetic pools that diverged before European colonization, and they further identified a set of genes linked with increased leaf and seed size and combined these results with quantitative trait locus data from Mesoamerican cultivars. The reference genome and the confirmation of dual centres of domestication provide very useful information when it comes to bean breeding and improvement programmes. Mamidi *et al*. (2016) conducted a Sequence-based introgression mapping and identified candidate white Mold tolerance genes in common bean. White Mold, caused by the necrotrophic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease of common bean with WM7.1 and WM8.3 as two quantitative trait loci (QTL) with major effects on tolerance to the pathogen being identified. Mamidi and his colleagues then concluded that, the most polymorphic candidate gene in the WM7.1 region encodes a BEACH-domain protein associated with apoptosis, while within the WM8.3 interval, a receptor-like protein with the potential to recognize pathogen effectors was the most polymorphic gene.

Comparative Transcriptome Analysis of resistant and susceptible common bean genotypes in response to soybean cyst nematode (SCN) infection was conducted by Jain *et al*. (2016) through gene expression profiling on common bean roots infected by SCN HG type 0 using next

generation RNA sequencing technology with two pinto bean genotypes, PI533561 and GTS-900, resistant and susceptible to SCN infection, respectively. Their results showed that genes encoding nucleotide binding site leucine-rich repeat resistance (NLR) proteins, WRKY transcription factors, pathogenesis related (PR) proteins and heat shock proteins involved in diverse biological processes were differentially expressed in both resistant and susceptible genotypes. This kind of studies provide very useful insights when it comes to disease management.

A genome-wide association study (GWAS) using a global Andean diversity panel (ADP) of 237 genotypes of common bean was conducted to gain insight into the genetic architecture of phenology, biomass, yield components, and seed yield traits, genotyped with 5398 single nucleotide polymorphism (SNP) markers (Kamfwa *et al*., 2015). Their study identified positional candidate genes, including *Phvul.001G221100* on *P. vulgaris* (Pv) chromosome 01, associated with days to flowering and maturity. Further, significant SNPs for seed yield were identified on Pv03 and Pv09 and localized with quantitative trait loci (QTL) for yield from previous studies conducted in several environments and contrasting genetic backgrounds. These results demonstrate the usefulness of GWAS in identifying agronomic traits in common bean. Another GWAS study was conducted to identify candidate loci underlying agronomic traits in a Middle American (Mesoamerican) diversity panel of common bean (Mogaddam *et al*., 2016). They used a panel of 280 modern bean genotypes from race Mesoamerica, referred to as the Middle American Diversity Panel (MDP), that were grown in four US locations, and a GWAS performed using 150,000 single-nucleotide polymorphisms (SNPs) for six agronomic traits. They discovered new and known genomic regions affecting the agronomic traits at the entire population, race, and location levels, and there were strong localized signals in a narrow genomic interval for three interrelated traits: growth habit, lodging, and canopy height.

Hoyos-Villegas *et al*. (2017) performed GWAS for drought tolerance and associated traits in common bean. Their GWAS study explored the genetic basis of variation for drought tolerance and related traits in a Middle American diversity panel comprising 96 common bean genotypes. Their panel was grown under irrigated and rain fed conditions and single nucleotide polymorphism (SNP) data were used to explore the genetic diversity and ancestry of the panel. They showed that estimations of genome-wide heterozygosity revealed that, on average, greater diversity is present in individuals with Mesoamerican (3.8%) ancestry, followed by admixed individuals (2.3%). They further identified 27 significant marker trait associations based on best linear unbiased predictors. These associations include seven markers for shoot biomass at harvest

under irrigation and five markers under rain fed conditions on *P. vulgaris* (*Pv*) chromosome Pv11, two markers for shoot biomass at flowering under irrigation on *Pv*02 and *Pv*08, two markers for seed size under irrigated and rain fed conditions on *Pv*09, seven markers for lodging score under irrigation on *Pv*02 and *Pv*07, one marker for leaf elongation rate on *Pv*03 and one for wilting score on *Pv*11.

Lastly, Rocha *et al*. (2017) developed a Rapid detection of *Macrophomina phaseolina* in common bean seeds using a visual loop-mediated isothermal amplification assay. The ideal conditions for detection were obtained in 45 min at 65 °C whereas the limit of detection was one infected seed per lot of 400 seeds. In summary as seen from the above findings modern technologies are being used to address both biotic and abiotic constraints in common bean production. The technique of Rocha *et al*. (2017) can also be used to foster material sharing by the different breeding programmes within regions or across regions, hence provider a wider germplasm for common bean improvement.

Chapter 3
Materials and Methods

3.1 Biophysical factors, farming systems, bean breeding and how they relate to the diversity of common bean, including landraces in Zambia

3.1.1 Study area

The study was conducted in four districts of Kasama, Mpika, Kafue and Lundazi in the Northern, Muchinga, Lusaka and Eastern provinces respectively. The four districts differ in altitude, rainfall, temperature, soils types and pH, and major farming systems (Eroarome, 2009; Mwale *et al*., 2007) that provided good diverse backgrounds for this study.

3.1.2 Selection of the different participants for the study

The selection of the participants was based on the different actors along the common bean value chain, from production to marketing and how these actors interplay to promote crop genetic diversity. The farmer groups considered for this study were those growing the four landraces in their respective four locations targeting their indigenous knowledge about the beans varieties/landraces, whereas the bean sellers were those situated at the Ntangali market at the Lusuantha border post between Malawi and Zambia to trace the element of cross border trade. The other participants included mainly the subject matter specialists such as the bean breeders, soil scientists, NGO staff, and the Seed Co (a private seed company) staff.

3.1.3 Research Design

This study was conducted to give a baseline information on a number of areas including: i) analysing the farming systems; ii) farmers' perception on the common bean landraces and their attributes along the value chain; iii) common bean breeders' opinions on the use of common bean landraces as parental materials in their breeding programmes; iv) soil scientists' opinions on the Zambian soils and how it impact common bean growing;, v) beans sellers' perception on the challenges on marketing common beans; vi) accessing data on the climatic information (rainfall, temperature, and relative humidity) from the meteorological department of Zambia. To facilitate comprehensive data collection, three data collection methods were employed namely focus group discussions, semi-structured interview, and the use of secondary data. These are discussed in detail below.

3.1.3.1 Group Discussions

Focus Group Discussions (FGDs) or simply group discussions (GDs) were held in two of the four study districts of Kafue and Lundazi districts with Shimabala and Mthilakubili farmer groups respectively. These farmer groups were of different sizes in terms of membership base (e.g. 43 members for Shimabala and 62 for Mthilakubili), sex (i.e. male and females), and age group (18-65 years old). These farmers were all registered as members of seed growers' associations in their respective districts. The minimum number of participants for GD with farmers was 6 and the maximum was 15, and the nearby non-members of these groups were welcomed to join the discussions. Another two GDs were held with common bean traders in Lusuntha, a border market between Zambia and Malawi, and Staff of Self Help Africa (SHA), Zambia office. For these two FGDs 8 and 5 participants participated for bean sellers and SHA respectively. Throughout these GDs, emphases were on their knowledge on common bean varieties in production including landraces with their particular traits of preference, factors that influence the choice of a variety to be grown by farmers, major production challenges, effects of seed colour and admixture on marketing, why admixture in production, levels of intercropping, crop rotation practices and the choice of the most important landraces in their locations. GD with SHA was mainly on their rationale for putting a lot of interests on these landraces of common beans.

3.1.3.2 Semi Structured interview

Semi-structured interviews were employed to allow the researcher to get an in-depth understanding of the common bean value chain and common bean landraces in the Zambian context based on the perception of the respondents. Louise Barriball and While, (1994) noted numerous advantages associated with semi-structured interview including: allowing for the clarification of interesting and relevant issues raised by the respondents, providing opportunities to explore sensitive issues, eliciting valuable and complete information, enabling the interviewer to explore and clarify inconsistencies within respondents' accounts, and helping respondents recall information for questions involving memory. However, for this study, it was considered for two main reasons; it's suitability for the exploration of the perceptions and opinions of respondents regarding complex and sometimes sensitive issues and enable probing for more information and clarification of answers, and the varied professional, educational and personal histories of the sample groups. The semi-structured interviews were conducted with three groups of respondents namely bean breeders and their technicians, soils scientists and their technicians, and seed companies workers. The aim was to seek respondents' opinion from public and private institutions with the view to shaping public-private partnerships.

Among the bean breeders, Dr Kennedy Muimui and Mr Robert Lungu of Zambia Agricultural Research Institute (ZARI), and Dr Kelvin Kamfwa and Mr Alex Mwape of the University of Zambia (UNZA) were interviewed. The interviews were centred on bean breeding objectives, the use of bean landraces in bean breeding, incorporating farmers in the breeding programmes, farmers' attitudes towards preferred traits of bean varieties. In addition, questions concerning new bean variety adoptions, key challenges in bean breeding, bean marketing of the new varieties, and the use of site specific bean varieties were part of the interview. Specifically, with the ZARI breeders, these were interviewed about the areas of the landraces, particularly where they were collected from, why these four landraces, how are they being grown by the different farmer groups, how do they safeguard aginst deliberate mixing by farmers, and challenges in growing these landraces.

To gain an in-depth understanding about the soils of Zambia, Professor Obed Isaac Lungu of UNZA, and Mr Raby Banda of ZARI were interviewed. The interviews focused mainly on the different aspects of soils of Zambia: soil types, soil nutrient levels, soil pH, soil organic matter level, soil toxicity, soil nutrient fixation, and soil management practices with particular interest in Kasama, Mpika, Kafue and Lundazi districts of Zambia that were growing the four landraces of common bean under investigation here.

Two staff from Seed Co, a private seed company, were interviewed with the aim of understanding their perception on the relationship between crop varieties and agro-ecologies, their priority crops, the reasons for not dealing in bean varieties, challenges and opportunities that exist for a private seed company including if any support from the government of Zambia.

In summary, all the interviews conducted were open-ended with prompts and probes being used during the interview process. They were also conducted in English and thus, there was no need for the services for an interpreter.

3.1.3.3 Use of Primary/Secondary data

During this study period in Zambia, from the Department of meteorology, I was given primary data on the climatic factors of rainfall, temperature, and relative humidity for the locations of Solwezi, Mbala, Mpika, Lusaka, and Lundazi over a three-year period (i.e. 2014- 2016). These correspond to the period during which the four landraces were being grown in the four locations. I was also given some secondary data in form of published shorthand books, and journal articles which were useful for my analysis and discussion. From ZARI through Kennedy Muimui of Bean Breeding department, I accessed two publications "Zambian Bean Varieties Descriptors",

and “the most popular Bean landraces of Zambia: the commandants of the bean market of Zambia”. Furthermore, ZARI, through Raby Banda of Soils department, I accessed a publication entitled “Participatory Village Development in Isolated Areas (PaViDIA) Field Manual (Volume 3) on Sustainable Agriculture Practices”. From the seed company, Seed Co, I accessed a brochure detailing maize hybrids in relation to Agro-ecologies of Zambia. In addition to these materials, additional materials were accessed online from two websites: http://www.bath.ac.uk/library/ and https://scholar.google.co.uk/ following my return from Zambia.

3.1.4 Statistical Analyses

The findings from the FGDs and interviews were summarised into different themes and presented under the result section. Nonparametric analyses mainly from ranking of characters and varieties/landraces of beans were performed in Minitab 17 Statistical Software (Minitab Inc, 2010) and PAST Software, Version 3.16 (Hammer *et al*., 2001) to measure the extent to which the different stakeholders value the different common bean varieties and traits. One-way analysis of variance procedure was used to test statistical differences in the rating of bean varieties and their traits among the four farmer groups in the four districts of the study using PAST3 software.

3.2 Molecular Marker Assessment of Genetic Diversity and Population Structure of Common Bean (*Phaseolus vulgaris*) Landraces from Zambia

3.2.1 The Plant Material

Four landrace populations from Zambia, four genepool control genotypes, and two Zambian cultivars from CIAT were used for this research study. The landraces were supplied to University of Bath by Self Help Africa (https://selfhelpafrica.org/uk/zambia/), a charitable organisation that works in collaboration with the Zambian Agricultural Research Institutes (ZARIs) to improve the livelihoods of communities through increasing common bean production and utilisation. The four landraces represent those that are grown in all bean growing communities of Zambia and they include Lusaka yellow, Lundazi, Mbala Mixture, and Solwezi with varying levels of seed admixture. The names of these landraces that were adopted for this study corresponded to the geographical locations in Zambia from which they were collected. The landraces were grown and maintained by small scale farmers using their traditional cultivation practices, from where they were collected by ZARI breeders, and maintained over three growing seasons (2014 to 2016) by different farmer groups. The bulked seeds of each landrace from the different locations for all the landraces were supplied to Self Help Africa who then forwarded them to the Crops Innovations, University of Bath, UK. While in the UK, the seeds were kept in the Plant Science laboratory,

Department of Biology and Biochemistry. The CIAT control genotypes were 'Diacol Calima' (G4494) from Colombia and 'Chaucha chuga' (G19833) from Peru for Andean genepool and 'ICA Pijao' (G5773) from Colombia and 'Chicharo' (G9794) from Mexico for Mesoamerican genepool. The CIAT reference G4494 showed two growth habits of Bush (later designated as G4494B) and semi climber (designated as G4494C) with white and pink flowers respectively. In addition to these four control genotypes, we also got two Zambian CIAT lines comprising of a landrace (G24493) and a commercial variety (G14470). Over 270 individuals of each landrace and 8 individuals of the CIAT Lines were selected randomly, germinated and used in this research study over the three growing seasons of 2014 to 2016. All the CIAT reference lines had been used in previous diversity studies for common bean and were reliable and effective at discriminating the two genepools (Asfaw *et al*., 2009; Blair *et al*., 2009b, 2010; Blair and Lorigados, 2016). Prior to planting, the bean seeds were soaked overnight in a moistened laboratory blue roll, and were planted and germinated on a compost manure of both fine, medium and coarse mixed in the ratio of 1:2:2 in a 8 cm diameter pot for each seed, in a tropical glasshouse set at a temperature of 20^{o}C, relative humidity of 70-80 percent, and 16 hours lighting regime. The seeds from the third growing season had the lowest germination percentage and planting was repeated three times to raise the required number of plants required for each landrace for this study.

3.2.2 DNA Extraction, and qualitative and quantitative determination

DNA was extracted from the first trifoliate leaf at 8 days from planting using the CTAB method described by Afanador *et al*. (1993) with a slight modification. Briefly, after grinding in liquid Nitrogen in a 1.5 ml Eppendorf tube, 300 µls of extraction buffer at room temperature was added and then heated in a water-bath set at 65^{o}C for 15 minutes. The rest of the remaining procedure is as described by Afanador *et al*. (1993). Again, the extracted DNA was dissolved in Sigma water instead of 1X TAE and its integrity evaluated on a 1% agarose gel, its concentration determined with a Nano Drop (Thermo Scientific, UK) and adjusted if necessary to 35-50 ng/µl.

3.2.3 Microsatellite markers and genotyping

3.2.3.1 Selection, Optimisation and PCR conditions

Fifty common bean microsatellite markers, both genomic and gene based, (Annex 1) from the BM, BMd, C, and Pv series were screened for their use in the Zambian germplasm characterization and diversity study from the sets developed by and/or used in Yu *et al*. (2000), Gaitan-Solis *et al*. (2002), Blair *et al*. (2003, 2006a, and 2009a), Buso *et al*. (2006), Burle *et al*.

(2010), and Wang *et al*. (2012). A sub-set of 28 microsatellites that were very polymorphic and discriminative with the Zambian common bean landraces were selected for use (Annex 2), of which 20 produced complete data sets and were considered for analysis (Table 3.1). Forward primers for each of the microsatellites were 5'-end labelled with either 6-FAM (blue), NED (yellow), VIC (green) or TET (red) fluorescent label dyes. The labelled forward primers and the unlabelled reverse primers were combined together in multiplexed PCR runs in two colour marker panels rather than the four colour panel as used by Blair *et al*. (2009a) due to differences in annealing temperature. Polymerase chain reactions included 22 µl of master mix (1X Reaction buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 5X loading dye and Nuclease free (Milliq) water), 1 µl (10 µM) Forward and Reverse Primers, 1 µl (35-40 ng/µl) of genomic DNA, and 1 U of *Taq* polymerase (New England Labs) for a final reaction volume of 25 µl on PTC 100 or PTC 200 (MJ Research) or SimpliAmp (Applied Biosystems) or T 100 (BIORAD) thermocyclers for all the markers. The thermocycling profile included a hot start at 92°C for 2 min, followed by 29 cycles of denaturation at 92°C for 1 min, annealing at 50-60°C for 1 min, followed by extension at 72°C for 2 min and a further primer extension at 72°C for 5 min and the final infinite hold at 10°C. Prior to allele visualisation in the automated capillary electrophoresis, 10 samples from each of the 96 well plates were randomly selected and run on a 2% agarose gel to confirm the amplification. Following the amplification of these PCR runs, the PCR were pooled together for each landrace to constitute a four colour marker panel ready for capillary electrophoresis.

Table 3.1 Details of microsatellite markers, their sequences and length (bp), optimum annealing temperature, expected and observed product sizes used in this study.

No.	Primer	Sequences	Length (bp)	Optimum annealing temp°C (Ta)	Expected product size[a]	Observed product size[b]
1	Pv-ag003	F: TCACGTACGAGTTGAATCTCAGGAT	25	57	167-171	162-164
		R: GGTGTCGGAGAGGTTAAGGTTG	21			
2	BMd32	F: ACACCCTTCATCTCCCTCAT	20	57	100-112	108-110
		R: ACCCATGTTGGATGTTGGAT	20			
3	BMd07	F: GGATATGGTGGTGATCAAGGA	21	57	168-171	167-183
		R: CATACCCAATGCCATGTTCTC	21			
4	PV-BR25	F: TAACATCAGACGCCGACGA	19	56	158	160-165
		R: GAGCTTCTCCGTCCTGTGT	19			
5	BMd18	F: AAAGTTGGACGCACTGTGATT	21	59	155-162	124-240
		R:TCGTGAGGTAGGAGTTTGGTG	21			
6	BMd03	F: TGTTTCTTCCTTATGGTTAGGTTG	24	57	187-228	105-227
		R:GTATCCTCCGATCAAATTCACCT	23			
7	Pv-gaat002	F: ACCTAGAGCCTAATCCTTCTGCGT	24	56.5	139	139-140
		R: GAATGTGAATATCAGAAAGCAAATGG	26			
8	BMd01	F: CAAATCGCAACACCTCACAA	20	54	172-200	200-202
		R: GTCGGAGCCATCATCGTTTT	20			
9	Pv-atgc002	F: AGCTTTCACACTATGACACCACTGG	25	59	134-150	140-154

		R: TGCGACATGAGAGAAAGACACGG	23			
10	Pv-ctt001	F: GAGGGTGTTTCACTATTGTCACTGC	25	56.5	152	139-173
		R: TTCATGGATGGTGGAGGAACAG	22			
11	BMd53	F: TGCTGACCAAGGAAATTCAG	20	56	108-112	103-107
		R: GGAGGAGGCTTAAGCACAAA	20			
12	BMd28	F: TGCATCAACTTTAGGAGCTTG	21	57	130-157	140-198
		R: TCTTGTCTTATCAGCAGGTGGA	22			
13	Pv-at006	F: CCGTTGCCTGTATTTCCCCAT	21	56.5	130-165	127-171
		R: CGTGTGAAGTCATCTGGAGTGGTC	24			
14	C119	F: CCACCATTGCTCTCAGTGTTA	21	57	251-292	270-374
		R: TAGATGTGTGTTTGTGTTCCG	21			
15	BM137	F: GGCTTACTCACTGTACGCACG	21	60	122-138	111-124
		R: CCGTATCCGAGCACCGTAAC	20			
16	BM211	F: ATACCCACATGCACAAGTTTGG	22	58	180-237	179-225
		R: CCACCATGTGCTCATGAAGAT	21			
17	Pv-tttc001	F: TTTACGCACCGCAGCACCAC	20	50	157-168	159-162
		R: TGGACTCATAGAGGCGCAGAAAG	23			
18	Pv-at007	F: GAAGAGTTGCAGATTGAGGT	20	54	190-216	404-443
		R: TTCTACCAGGCAAATATTGAG	21			
19	Pv-gat001	F: AGTGGTGTGGATGCTGTTGTT	21	56.5	183	188-256
		R: GGCGCTGAGATCAGTAGGAG	20			
20	BM33	F: TACGCTGTGATGCATGGTTT	20	58	110-120	80-107
		R:CCTGAAAGTGCAGAGTGGTG	20			

[a]expected product sizes are those reported in other earlier studies using these same markers, and [b]observed product sizes in this study by these markers.

3.2.3.2 Agarose gel electrophoresis and band size estimation during SSR Screening

DNA was isolated from nine individuals of each landrace form 2014 seed lot and one from each of the CIAT reference lines as in the above method. PCR was run using these DNA samples for all the 50 SSR markers. The PCR products were run on 3% agarose gel at 70 volts for 90 minutes. The PCR products were loaded alongside the 100bp DNA ladder on both extreme gel well lines, and their sizes compared.

3.2.3.3 Capillary Electrophoresis

The pooled four-colour panel markers of PCR products resulting from multiplexing two non-overlapping panels based on expected allele size labelled with four different dyes were prepared. A master mix of 10 µl consisting of 4 µl of each panel pooled PCR product, 5.7 µl of formamide (Hi-Di) and 0.3 µl of GeneScan-500 LIZ standard (Applied Bioscience Inc, USA) was prepared in a 96-well Micro Amp Plates (Applied Bioscience Inc, USA) to represent each individual plant for capillary electrophoresis. Fragment separation was performed using ABI PRISM 3730 automated fragment analyser (Applied Biosystems Inc, USA) at Source Bioscience Laboratory, Nottingham, UK, which generated .fsa files representing each fragment size(s) for each individual within the colour panel for subsequent analysis.

3.2.3.4 Allele calling

Alleles were automatically called on the basis of band sizes for each microsatellite and the different individuals using Fragman Program of R software v. 3.1.2 as described by Covarrubias-Pazaran (2016). A data matrix of individual genotypes by SSR markers was generated in Microsoft Excel 2013 and converted to a .txt file with rows encoding allele sizes for each individual and columns that encoding allele sizes for each microsatellite.

3.2.4 Analysis of genetic diversity using R Package, GeneAlex and STRUCTURE software

The data matrix of individuals of each landrace by different band sizes and for the different SSR markers were transformed to a binary data for the SSR screening data and their clustering patterns and genepool association analysed using R software. The preliminary results from this screening led to the upscaling of this study and the massive data was analysed as described here under. The polymorphic level of SSR markers and genepool association of these landraces to the test genotypes was evaluated by analysing the different individuals in the twelve populations comprising of 4 landraces, 6 CIAT reference lines, and 2 sub-population that resulted from the hybridization of G4494 (G4494B and C), a reference CIAT line. The genetic statistics based on twelve populations and 1101 individuals were calculated using GenAlex 6.5 Software (Peakall and Smouse, 2006 and 2012) including polymorphic number of alleles, effective number of alleles, observed, expected and unbiased heterozygosity, allelic frequencies, inbreeding coefficient, Analysis of molecular variance (AMOVA) and principal coordinate analysis (PCA). Polymorphism information content (PIC) was calculated using the formula developed by Anderson *et al*. (1993). Population structure was determined with 5 independent runs in STRUCTURE v. 2.3.4 software (Pritchard *et al*., 2002 and 2003; Falush *et al*., 2003) assuming an admixture model and no *a priori* population assignment for genotype classification. *K* values ranged from 2 to 16 were run at 50,000 burn-ins and 100,000 repetitions. The bar-plot function was used to display the *Q* values for each genotype within each of the subpopulations (K), and the ideal *K* value was determined by an Evanno test performed in the Structure Harvestor of Earl and vonHoldt (2012).

3.3 Agro-morphological characterisation and genetic diversity of common bean (*Phaseolus vulgaris*) landraces from Zambia

3.3.1 Plant materials and growth conditions

In total, four populations of landraces of Lusaka yellow, Lundazi, Mbala mixture and Solwezi from Zambia, four genepool control genotypes (G4494B, and C – Andean, and G5773 - Mesoamerican) and a commercial variety (G14470) from Zambia from CIAT, were used for this research study. The source of the seed supplied and its composition, growth medium and condition were as described under 3.2.1. Exceptions here are that, 5 litres pots were used and the compost mixture was supplemented with a slow releasing nutrients Osmocote Extract (Standard 12-14M, ICL, UK) at the rate of 4 grams per litre. Osmocote Extract is a compound NPK (Mg) fertilizer with micro nutrients (Boron, Manganese, Copper, Zinc, Iron, and Molybdenum) included to provide nutrients over the entire growth cycle of the plants especially form ported plants.

3.3.2 Experimental Design

Twenty plants of each landrace and ten plants for the CIAT materials were grown from randomly selected seeds and used for morphological data collection. Experimental units consisted of two rows of 10 for each landrace and two rows of 5 plants of each CIAT materials, with intra- and inter-row spacing of 15 cm and 50 cm, respectively. This was replicated three times to represent the three seed lots received from Zambia over three growing seasons of 2014 to 2016.

3.3.3 Data collection procedure and methods

Thirty morphological descriptors (14 quantitative and 16 qualitative) of common bean (Table 3.2) were evaluated according to the agro-morphological descriptors used by Hornakova *et al*. (2003), Hegay *et al*. (2013), Lima *et al*. (2012)and Ligarreto and Martinez (2014) for *P. vulgaris*. The variation on seed yields and seed parameters were considered important for this study due to their importance for common bean breeding programs, and further owing to the fact that they are less affected by environmental factors (Zizumbo-villarreal *et al*. 2005; Mercati *et al*., 2013; Awan *et al*., 2014). Qualitative morphological traits were binary coded as 1 for presence or 0 for absence for each individual plant (e.g., hypocotyl colour green: presence (1) or absence (0); hypocotyl colour red: presence (1) or absence (0) and hypocotyl colour pink: presence (1) or absence (0) since common bean is mainly a self-pollinated crop, there is no chance of finding heterozygotes. For quantitative characters, measurements were recorded at different stages of

bean plant's growth, from seed emergency through flowering to seed harvest on randomly selected 10 plants each landrace and CIAT reference line.

Table 3.2 Morphological qualitative and quantitative variable evaluated amongst the common bean landraces from Zambia

Qualitative Morphology	Quantitative Morphology
Hypocotyl colour	Number of days to flowering
Stem colour	Number of days to pod maturity
Colour of leaf venation	Seed length (mm)
Plant growth habit	Seed width (mm)
Flower colour	Seed height (mm)
Dry Pod colour at maturity	Seed volume (mm^3)
Pod break position	Leaf length (cm) at flowering
Pod curvature	Leaf width (cm) at flowering
Position of pods on the plants	Leaf stalk length (cm) at flowering
Seed shape	Internode length (cm) at flowering
Seed colour	Average number of pod per plants
Brilliance of the seed	Pod length (cm) at maturity
Seed prevalent	Pod width (cm) at maturity
Presence of helium ring	Number of seeds per pod
	Weight of 100 seeds (gm)
	Weight of seeds per plant (gm)

3.3.4 Analysis of morphological diversity parameters using PAST3, POPGENE, and STRUCTURE Software

For qualitative traits, data was summarised on the presence (1) and absence (0) for each qualitative trait scored, a matrix of binary data was generated for all the traits, and individuals of the different landraces studied, and used in the subsequent analysis. Univariate and multivariate analysis were performed in PAST Software, Version 3.16 (Hammer *et al*., 2001) to generate both Dendogram and principal component analysis (PCA) using these Agro-morphological traits as a mean of quantification of genetic divergence among these landraces. The genetic diversity

parameters using these qualitative traits such as number of alleles (Na), effective number of alleles (Ne), private number of alleles, Nei's gene diversity (h), and Shannon's information index (I) were calculated for each qualitative trait, and the landraces using the program POPGENE, version 1.31 as described by Yang and Yeh (1993). The software STRUCTURE, as described by Pritchard *et al*. (2000; 2003), was used to study the population structure of the Zambian landraces using the fore mentioned qualitative traits; and a combination of both qualitative and quantitative traits. For the structure analysis, the admixture model was used with 5,000 burning periods and 50,000 repetitions to estimate each K value, with 15 independent runs from K = 1 to 15. The bar-plot function was used to display the *Q* values for each genotype within each of the sub-populations (K), and the ideal *K* value was determined by an Evanno test as described in Earl and vonHoldt (2012).

For Quantitative traits, the summarised data (means of the different traits measured from 10 plants/pods/seeds) were entered into a Microsoft Excel 2013 file, and analysed using Minitab 17 statistical software (Minitab Inc, 2010) and PAST Software, Version 3.16 (Hammer *et al*., 2001) for descriptive analysis (DA), correlations and Analysis of variance (ANOVA). DA was used to distinguish between accessions and divide them into groups based on morphological traits. Dytham (2011) had shown that, DA grouped accessions with typical characters, and estimated the correct and incorrect percentage of classifications and maximizes differences between classes while minimizing those within classes, which is different from the PCA. In the PAST software and R Software, qualitative and quantitative data were used to produce both the Dendogram and PCA for each data set and a combination of all the data as described by Hammer *et al*. (2001) and R Core Team (2013) respectively. However, for quantitative traits, the data were first transformed before analysis as described by Peng *et al*. (2007). The PCA was also used to analyse the variability amongst the different landraces as well as to identify the optimum number of morphological traits which explain a high proportion of the variability.

3.4 Determination of the macro and micro element concentrations from the common bean landraces from Zambia

3.4.1 The plant materials

For this analysis, the different sub-populations under each landrace were treated differently, as detailed here under (Table 3.3). A total of 50 samples were used for this study, that is, Lusaka yellow had 8 sub-populations, Lundazi had 8 sub-populations, Mbala mixture had 7 sub-

populations, and Solwezi had 8 sub-populations. Additionally, there were sub populations that overlapped between landraces: 1 overlap for Lusaka yellow and Mbala mixture, 2 for Lundazi, Mbala mixture, and Solwezi, and 8 for Mbala mixture and Solwezi, totalling to 42 sup-populations from all the landraces. In addition to these landrace sub-populations, there were four Zambian-Malawian varieties of Katwetwe, Kabulangeti, Sugar beans, and White bean that were collected from the Lusuntha border market between the two countries due to their market dominance, and finally the 4 CIAT reference varieties of G5773, G4494A, G4494C, G14470) were included in this study.

Table 3.3 Details of the sub-populations of common bean with their seed coat colour and 100-seed weight used for the macro and micro mineral concentration determination

Landrace	Sub-pop	Seed Colour	100SW
Lusaka Yellow (LY)	LY1	Yellow	27.05
	LY2	Creamy yellow	29.47
	LY3	Deep yellow	34.52
	LY4	Olive green	48
	LY5	Yellow	31.26
	LY6	Creamy yellow	24.31
	LY7	Brownish yellow	29.51
	LY8	Yellow	26.91
Lundazi (LU)	LU1	Dark red	42.54
	LU2	Maroon	20.75
	LU3	Red	22.51
	LU4	Dark brown	33.58
	LU5	Black	24.27
	LU6	Purple	41.17
	LU7	Red mottled	27.94
	LU8	Purple	25.19
Mbala Mixture (MM)	MM1	Pinkish mottled	52.68
	MM2	Purplish mottled	30.67
	MM3	Grey to brownish green	38.29
	MM4	Yellow	31.45
	MM5	Yellow	26.9
	MM6	Pinkish with yellow speckles	50.15
	MM7	Brownish yellow	30.44
Solwezi (SO)	SO1	Pinkish mottled	51.94
	SO2	Purple mottled	32.16
	SO3	Dark Red/Maroon	40.16
	SO4	Pink	20.55
	SO5	Red	30.01
	SO6	Red mottled	37.4

	SO7	Pink with black mottled	38.5
	SO8	Pink with black speckles	30.07
LY and MM overlap	LYMM	Yellow	37.51
LU, MM and SO overlap	LMS1	Purplish with speckles	39.21
	LMS2	Grey to brownish green	29.23
MM and SO overlap	MS1	White	31.83
	MS2	Pinkish mottled	31.57
	MS3	Purplish mottled	29.41
	MS4	Brownish to Pinkish mottled	27.02
	MS5	Dark grey with speckles	37.72
	MS6	Blackish with brown mottling	38.52
	MS7	Dark brownish mottled	49.74
	MS8	Black with Cream mottling	33.16
CIAT Reference Lines	G5773	Black	19.76
	G4494B	Red mottled	39.7
	G4494C	Red mottled	31.89
	G14470	Cream purple stripped	36.91
Zambian/Malawian commercial Lines	Long White	White with purple dotted helium	36.35
	Kabulangeti	Dark grey speckled	46.16
	Katwetwe	Purple mottled	30.81
	Sugar Bean	Cream purple stripped	37.56

3.4.2 Sample preparation and Acid digestion

The 100-seed weight of each of the 50 sub-populations were determined prior to sample preparation as indicated in Table 3.3 above. Six grams of sample were ground into a fine powder using the Andrew James wet and dry grinder (Andrew James, UK Ltd). Two grams of finely ground bean flour were stored in the boiling tubes for subsequent acid digestion. Acid digestion was conducted as described by Tryphone and Nchimbi-Msolla (2010) and Alzahrani *et al*. (2016). Briefly, to the fine ground bean flour in each of the boiling tubes was added 20 ml of 6M 70% nitric acid for trace element analysis (Sigma-Aldrich, UK) and allowed to stand for an overnight. The boiling tubes were then arranged vertically in the glass beaker and placed in Heraeus B6 Incubator (Fisher Scientific, UK) at a temperature 50^0C for 3 hours. The digested samples were cooled to room temperature, filtered with Whatman filter paper, and diluted with trace element grade deionised water (Sigma-Aldrich, UK) in 25 ml volumetric flask to the mark. The solution was thus ready for determination of macro and micro elements in the atomic absorption spectrophotometer (AAS) method of Perkin-Elmer 2380 (Fisher Scientific, UK).

3.4.3 Determination of micro and macro elements in the samples

Calcium (Ca), Magnesium (Mg), Copper (Cu), Potassium (K), Manganese (Mn), Phosphrous (P), Iron (Fe) and Zinc (Zn) were quantified using Atomic Absorption Spectroscopy (AAS) method of Perkin-Elmer 2380 (Fisher Scientific, UK), and their concentrations were converted and expressed in $mg100g^{-1}$ from the absorbance read in the (AAS) (Maraghan and Grafton, 2001; Akond *et al*., 2011; Gouveia *et al*., 2014). For Iron and Zinc, the readings were evaluated against the standard curves prepared from the Iron diluted to a concentration of 100 mg l^{-1} and Zinc diluted to 50 mg l^{-1} (Blair *et al*., 2009c).

3.4 4 Data analysis

Data were collected in triplicates and the averages were computed in Microsoft Excel 2013. A Shapiro-Wilk's test was performomed in the R software to test for data normality. One-way ANOVA was applied to evaluate the variance of these micro and macro elements parameters; the Pearson coefficient was used to verify the existence of statistically significant correlations among the variables; the multivariate analysis of main PCA components was performed, with the aim to detect the existence of clusters grouping amongst the bean accessions according to their mineral concentrations. All these analyses were run using PAST software, Version 3.16 (Hammer *et al*., 2001) and R Software (R Core Team, 2013).

Chapter 4

Biophysical Factors, Farming Systems, Bean Breeding and how they relate to the Diversity of Common Bean, including Landraces in Zambia

4.1 Introduction

The Republic of Zambia, is a landlocked country in Southern Africa, neighbouring the Democratic Republic of the Congo to the north, Tanzania to the north-east, Malawi to the east, Mozambique, Zimbabwe, Botswana and Namibia to the south, and Angola to the west (Chomba, 2004). The country has a total land area of 743,390 Km^2 (287,024 sq. miles), current population of 17,344,385 hence a population density of 23 per Km^2 (60 people per mi^2), with 40.5 % of her population living in the urban areas (Worldmeters Statistics, 2017). Zambia's Gross Domestic Product (GDP) is $19.55 Billion, GDP per capita of $1,178.39, and has a life expectancy of 60.79 years (FAOSTAT, 2016). By its location, Zambia is actively doing business with both countries from the Eastern coast of the Indian Ocean, and the western coast of the South Atlantic Ocean with the main economic activities being mining, agriculture, tourism, gemstone mining and hydro power, amongst others (Eroarome, 2009). Agriculture is of key importance to Zambia, for which crop and livestock diversity are essential. Linhart and Grant (1996) reported toxic soils, fertilizers, mowing and grazing, soil moisture, temperature, light intensity, pollinating vectors, parasitism, gene flow, and natural dynamics as other factors that affect diversity and population differentiation in crop species. Furthermore, considering its vast land size, and low population density, Zambia experiences a lot of climatic, edaphic, and farming system variations that could shape crop diversity, including that of the common bean.

Zambia experiences a predominantly sub-tropical climate characterised by three distinct seasons: a hot and dry season (mid-August to the end of November), a rainy season (November through to April), and a cool dry season (May to mid-August). Rainfall is strongly influenced by the movement of the Inter-Tropical Convergence Zone (ITCZ) as well as the El Nino/Southern Oscillation (ENSO) phenomenon (Chomba, 2004; UNDP, 2010) and varies from an annual average of 600 mm in the lower south up to 1300 mm in the upper north of the country. Furthermore, Phiri *et al.* (2013) in their review on adaptation of Zambian agriculture to climate change noted that there is an increasing trend in temperature and a decreasing trend in rainfall from the period 1980 to 2011. Based on rainfall patterns and soils, Zambia has three Agro-ecological zones: I, IIa, IIb and III (Chomba, 2004; Eroarome, 2009). A report presented by Ndiyoi *et al*. (2007) on the Baseline study on the food crop diversification support project

(FoDiS) in drought prone region I and II of Zambia, demonstrated the need to establish a more efficient and cost effective multiplication and distribution system for root and tuber crops planting materials by the organizations promoting cassava and sweet potatoes in these drought prone regions, and that dependence on rain fed agriculture is the main cause of the variation in food production, and prices in Zambia.

Five major farming systems have been identified in Zambia: shifting cultivation, semi-permanent hoe system, semi-permanent hoe and ox plough system, semi commercial cultivation, and commercial systems (Chomba, 2004), and the smallholder households are mainly associated with the first four farming systems whilst the large-scale farmers are largely associated with the latter farming system. These farming systems take on different form of soil and water management, and the concentration of major crops differ from farming system to farming system (Eroarome, 2009). A comparative study on conservation and conventional agriculture among smallholder farmers in Zambia showed that conservation agriculture, as currently practiced does not reduce the labour required during critical periods of the farming cycle (Umar *et al*., 2012). Mutuo *et al*. (2012), further showed different rate of water infiltration into the soil existed between conservation and conventional practices of agriculture. Additionally, with increasing land constraints in most areas, fallow periods have drastically declined from a range of 15-20 years to an average of 3 years or less (Kwesiga *et al*. 2003). The traditional farming systems that farmers have previously employed to sustain their productivity cannot any longer effectively work due to population pressures. Farmers have continued to perceive a decline in soil productivity, and continued water shortages in low rainfall areas (Chomba, 2004), that affects both crop productivity and diversity equally. This implies, the soils are being subjected to very intense farming practices, and thus the need to access its impact on crop diversity.

Despite the above climatic, edaphic and farming systems variations, agriculture remains a dominant economic activity in Zambia, with about two thirds of households being agricultural and agriculture is the most important livelihood strategy by most of the people (Hamzakaza *et al*., 2014). Potential arable land covers 47 % of the country's total land but only 15% of this is under cultivation (Eroarome, 2009), and the crop land is estimated at 7.08 %, with the major crops being Maize (*Zea mays*), Sorghum (*Sorghum bicolor*), Rice (*Oryza spp*.), Finger millet (*Eleusine coracana*), Wheat (*Triticum aestivum*), Barley (*Hordeum vulgare*), Cassava (*Manihot esculenta*), Sweet Potatoes (*Ipomea batatus*), Potatoes (*Solanum tubersum)*, Groundnuts (*Arachis hypogaea*), Soybean (*Glycine max*), Common bean (*Phaseolus vulgaris*), Sunflowers (*Helianthus annuus*),

Sugarcane (*Saccharum spp.*), Tobacco (*Nicotiana tabacum*), Coffee (*Coffea spp.*), Tea (*Camellia sinensis*), Cotton (*Gossypium spp.*), Bananas (*Musa spp*), Tomatoes (*Solanum lycopersicum*), Oranges (*Citrus sinensis*), amongst others (FAOSTAT, 2015 and 2016).

The promotion of agriculture and the above major crops, as well as extension information to farmers, is headed by the Department of Agriculture (DoA) in collaboration with Zambia Agricultural Research Institute (ZARI), Southern African Bean research Network (SABRN), Plan International, Self Help Africa (SHA) and World Vision International. These different actors play different roles, for instance, DoA is a policy making body and general coordination of agricultural activities, the ZARI conducts on-farm research trials and demonstration plots to evaluate and select suitable varieties with farmers, and other development partners conduct dissemination of technologies that are crop specific in nature in several communities in the different provinces of Zambia (Hamzakaza *et al*., 2014). Equally, the funding of Agricultural activities is key to the success of the agricultural activities, as well as adoption of a new technology, thus, Ndiyoi *et al.* (2007) noted government, donors, private sectors and NGOs as key sources of funding. It is imperative to mention here that the agricultural development in Zambia is an interplay between the public and private institutions, thus, public-private partnerships with the farmers are at the forefront of all the planning phases.

Agriculture being a key economic activity in Zambia with different key actors in both the public and private institutions, and that there is a lot of variations being observed in soils, climate and farming systems, this study was conducted to provide a baseline information to the use of common bean landraces in Zambia, and factors that might contribute to their genetic diversity and population structure. Specifically, this study was aimed at addressing the following questions:

i) How did the ZARI bean breeders collect and maintain the common bean landraces over different years?
ii) What characters/traits are important for both bean farmers, breeders, and consumers?
iii) What is distinctive about the four common bean landraces across the four project sites?
iv) Besides these landraces, do farmers and sellers of beans know the varieties that they grow and their sources?
v) What factors affect the marketing of beans in Zambia across the study locations?
vi) How often do the ZARI and UNZA bean breeders use landraces in their breeding programmes; and what breeding objectives are being addressed?

vii) How do the farming systems, climatic factors, and soil factors differ across the four project sites in Zambia?

viii) What roles do the private institutions play in promoting diversity of common bean?

Addressing the above questions was aimed at providing in-depth knowledge based on the perception of the respondents that is needed to identify and characterise the common bean landraces from Zambia, which was the main objective of this entire study. It is further anticipated that some of the biophysical factors being investigated are directly linked and useful in providing explanations on the level of variation in the common bean landraces over the four study locations in Zambia. During the group discussions with farmers and interviews with bean breeders and soil scientists, each objective was discussed independently and participants would agree on what they consider as a final response for each objective. Where the responses were not clear, the questions were rephrased or probing was done to get a response that suits the question under discussion.

4.2 Results

4.2.1 Collection and Maintenance of Common bean landraces by ZARI breeders

During the semi structured interview with Kennedy Muimui and Robert Lungu of ZARI, it was confirmed that, the four common bean landraces were collected from Solwezi, Mbala, Lundazi, and Kafue districts of Zambia, and hence their origin of collection were maintained as their name plus some description such as Mbala mixture, to mean a landrace from Mbala with mixed populations. The collection from Kafue district was called Lusaka yellow due its location within the Lusaka province. The two ZARI bean breeders further mentioned that these four districts share international boundaries with other countries: Solwezi boarders the Democratic Republic of Congo (DRC), Mbala - the Republic of Tanzania, Lundazi – Malawi, and Kafue – Zimbabwe.

Mr Robert Lungu explained that, for the last three to four years, these landraces were being maintained by different farmer groups and at Kasama Research Station. The seeds of these landraces were distributed by ZARI to the selected groups (Table 4.1) during the planting time, and at harvest, the seeds from the different farmer groups were bulked together landrace by landrace for all the four landraces by ZARI team and kept for the subsequent distribution for planting in these locations again. The farmer groups are trained with proper agronomic practices for common bean production to maintain uniformity of farming practices. No fertilizer was applied to the soil during the growing periods. Planting dates over the different growing seasons are presented in Table 4.1 below:

Table 4.1 Farmer group/Site, District, Province, and the planting dates during the growing of the landraces

No.	Group Name/Site	District	Province	Planting date per season		
				2014/2015	2015/2016	2016/2017
1	Misamfu Research	Kasama	Northern	13/01/2015	14/01/2016	16/01/2017
2	Shangila	Mpika	Muchinga	23/01/2015	19/01/2016	12/01/2017
3	Shimabala seed growers assoc.	Kafue	Lusaka	21/01/2015	18/01/2016	16/01/2017
4	Mthilakubili seed growers assoc.	Lundazi	Eastern	20/01/2015	05/01/2016	17/01/2017

Due to limited resources, the maintenance of these landraces were excluded from Solwezi and Mbala districts due to long distances involved to reach these sites that would impact on monitoring.

4.2.2. Farmer groups' and sellers' perception and knowledge about the bean landraces/varieties value chain

Group discussions (GDs) with individual farmers and farmer groups have revealed important aspects regarding the common bean as a crop and the responses were documented as the following:

4.2.2.1 Common bean characters and their importance by locations

GDs with farmers in Kafue and Lundazi districts revealed varying preferences in common bean characters that are considered in these locations (Table 4.2). These characters further affect bean breeding as the breeders need to pay particular attention to most of these characters in each single variety to be released.

Table 4.2 Key common bean characters considered for the adoption of new varieties in Kafue and Lundazi. 1= (Less important), 2= (Intermediate), and 3= (Very important).

Key bean character	Kafue	Lundazi	Remarks
Pod length	3	3	Farmers from the two locations have learnt to associate these character to high yields of the different bean varieties
No. of pod/plant	3	3	
Seed/pod	3	3	
Yield	3	3	Used as ultimate measure for a variety against all the production challenges
Seed size	2	2	Seed size and shape are intermediate in all locations while seed colour is very important; Kafue prefers yellow seed colour and Lundazi prefers red seed colour
Seed Shape	2	2	
Seed colour	3	3	
Plant height/growth habit	1	1	Less important because even climbing varieties are not provided with stakes to climb on, and yet they differ in terms of yields
Early maturity	3	3	Very important at the household level
Green leaf consumption	3	3	Very important since green leaves are eaten as vegetables
Seed culinary qualities	3	3	Very important as tend to be associated by colour for these two locations
Drought resistance	2	2	Intermediate to very important as they contribute to the over yields of the different varieties. Both field and storage pests were recognised for the two locations too.
Disease resistance	3	3	
Insect pests resistance	3	3	
Storage life	3	3	Important particularly where farmers have to bulk their produce and sell later.
Market value	3	3	Closely linked to seed colour

4.2.2.2 Common bean agronomic practices and challenges by locations

A number of key agronomic practices, production and marketing challenges were discussed during the GDs, and key amongst which are summarised by locations (Table 4.3). The discussions included bean farmers and sellers from the two districts of Kafue and Lundazi.

Table 4.3 Key agronomic practices, production and marketing challenges in Kafue and Lundazi districts of Zambia. Yes = practiced or present and No = Not practiced or absent

Agronomic practice / production/marketing challenges	**Kafue**	**Lundazi**	**Remarks**
Proper site selection for common bean production	Yes	Yes	Farmers of all locations associated proper site selection with good yields
Choice of the seed materials*	Yes	Yes	Source of the planting materials depends on the availability and the purpose of production. Seed production is mainly from ZARI or NGOs like SHA
Planting date(s)	15-20th of Jan	January to March	Narrow range in Kafue (Restrictive) while it has wider range in Lundazi (Relaxed)
Intercropping	Yes	Yes	Common in all locations with maize as the intercrops. Seed crops are not intercropped in all locations
Fertilizer application	Yes	No	NPK and Urea are in Kafue while no fertilizer in Lundazi
Spraying	Yes	Yes	Diathene is used in Kafue for field pests, while burnt ashes is used in Lundazi for storage pests
Crop rotation	Yes	Yes	Practiced in all sited although the fallow period has shortened in all locations during the rotation.

Pest and diseases challenge	Yes	Yes	Both field and storage pests are recognised
Marketing challenge	Yes	Yes	Mainly resulting from low production, thus farmers under the associations have to bulk their produce before marketing

*the planting materials are sourced from ZARI, NGOs e.g. SHA, local markets like Soweto in Lusaka and Ntangali market in Lusuntha border post in Lundazi, and from the neighbouring farmers.

4.2.2.3 Production of the common bean landraces over the study periods

Another GD was tailored towards the landraces of common beans that were being grown by individual farmers have revealed variations in their preferences in yields and marketing opportunities. The yield and marketing of these common beans were location specific except for Solwezi with the highest yields in Kafue, meanwhile Mbala mixture has the lowest yields and marketing opportunities in these two locations (Table 4.4).

Table 4.4 Yield and marketing rankings for common bean landraces in Kafue and Lundazi. 1 = highest yield and high marketing opportunity. 1 = highest yield and high marketing opportunity, whereas 4 = lowest yields and lowest marketing opportunity

Yield and Marketing	Landraces	Kafue Ranking	Lundazi Ranking
Yields	Lusaka yellow	2	2
	Lundazi	4	1
	Mbala mixture	3	4
	Solwezi	1	3
Marketing	Lusaka yellow	1	2
	Lundazi	2	1
	Mbala mixture	4	4
	Solwezi	3	3

In addition to the bean characters (Table 4.2) and the landrace preferences (Table 4.4) above, common bean traders were asked whether they are aware of the names and sources of the varieties in the market (Figure 4.1). The bean sellers in Ntangali market in the Lusuntha border post provided detailed names of the varieties that were available in the market on the discussion day, and they went on to mention whether they were Zambian or Malawian varieties.

Surprisingly, Lundazi landrace that is grown in the border district of Lundazi came out as a variety from Malawi not Zambia, and that it's a commercial variety not a landrace in Malawi. This is one case to show how African porous borders can lead to germplasm exchange without following legal procedures in place normally required for such material exchange. This porous borders mainly result from the fact that the same ethinic group live on the either side of the borders hence making the movement of farm inputs including seeds very easy from one country to another.

Figure 4.1 Focus group discussions in Kafue (A) and Lusuntha boarder market (B) in Lundazi with bean producers and sellers respectively. Photo Credit: Mr Robert Lungu and Alex Abaca respectively.

4.2.3 Use of common bean landraces in bean breeding and the breeding objectives being addressed by these breeders

Semi-structured interviews with the common bean breeders from ZARI and UNZA confirmed that the landraces are being used in the current common breeding programmes. The ZARI breeders under the leadership of Dr Kennedy Muimui are using mainly Lusaka yellow to address the problems of low yields and diseases, particularly leaf angular leaf spot whose causative agent is a fungus (*Phaeoisariopsis griseola*). The UNZA bean breeders under the leadership of Dr Kelvin Kamfwa are using Solwezi, Lusaka yellow, and Kabulangeti (another landrace not used in this study) targeting the problems of low nitrogen fixation, bean bruchid (*Acanthoscelides obtectus*) insect pest, and other agronomic traits (low yields and drought tolerance). Dr Kelvin acknowledged that, the lack of well characterised common bean landraces/varieties is a big problem that forces them to use these unknown landraces.

However, Dr Kelvin Kamfwa further noted that the landraces of common bean contain mixed populations that are useful in the bean breeding programmes but require the use of a single seed

as a start up breeding material to achieve reproducibility of the breeding activities. This observation was based on the fact that landraces contain mixed populations with diverse levels of performance under different agro-ecological zones; therefore, it can be very difficult to come back to the same landraces and sample the seed types if you do not start from a single seed. Both breeders stressed the importance involving farmers at the different stages of the common bean breeding program, as this made acceptability of the new varieties to be very high among farmers who could have been involved in its evaluation during the participatory evaluations. Dr Kennedy Muimui mentioned that, farmers have associated certain agronomic traits to good bean varieties, for instance, long bean pods to high yields. One other challenge that came from both institutions, is that farmers do not provide stakes for their climbing varieties of beans which reduces greatly on the yield potential of these climbing varieties. The key reason for this refusal is the extra work that comes with staking when there are competing demands for labour elsewhere during the same production period.

4.2.4 Variation in farming systems, climatic and edaphic factors in selected locations in Zambia

The variation in farming systems, climatic and edaphic factors were investigated during the focus group discussions and semi structured interviews across locations. The primary climatic data was accessed from the Zambian meteorological department. The results are presented here under:

4.2.4.1 Variation in the farming systems

The Zambian farming systems was reported to fall under five major systems of: shifting cultivation, semi-permanent hoe system, semi-permanent hoe and ox plough system, semi-commercial cultivation, and commercial systems (Chomba, 2004; Eroarome, 2009). These farming systems mainly used two cultivation systems of Conventional Tillage (CT) and Conservation Agriculture (CA). The former being dominant over the latter, and their details provided in Mwale *et al*. (2007). However, during group discussions with farmers and farmer groups and semi-structured interviews with other key informants revealed three common farming systems including: small scale, medium scale, and commercial farming systems. The discussions and interviews revealed that, this classification tends follow the size of the land holdings, soil management practices, level of inputs and anticipated benefits. The respondents further noted that CA currently dominates among medium scale farming systems, and it is present in an equal rate in the small scale and commercial farming systems. This observation was the same for all the locations for the study. The respondents also pointed out that, the practices of using crop mixture

in the same field is greatest with small scale farming through medium farming and lowest in commercial farming.

4.2.4.2 Variation in the climatic factors

Monthly averages for climatic raw data for rainfall, relative humidity (RH), and minimum and maximum temperatures across the locations of Kafue, Lundazi, Mbala and Solwezi over the different growing seasons (2014 to 2016) were accessed from the meteorological department of Zambia. These raw data were further processed, and the averages for January, February and March for each year over the locations are presented (Figures 4.2, 4.3 and 4.4). The three months were chosen because they are the common bean growing months in Zambia. For relative humidity, the data was not complete for all the locations over the years, and therefore, it is only the data for Solwezi that are presented here to show the trend in its variation.

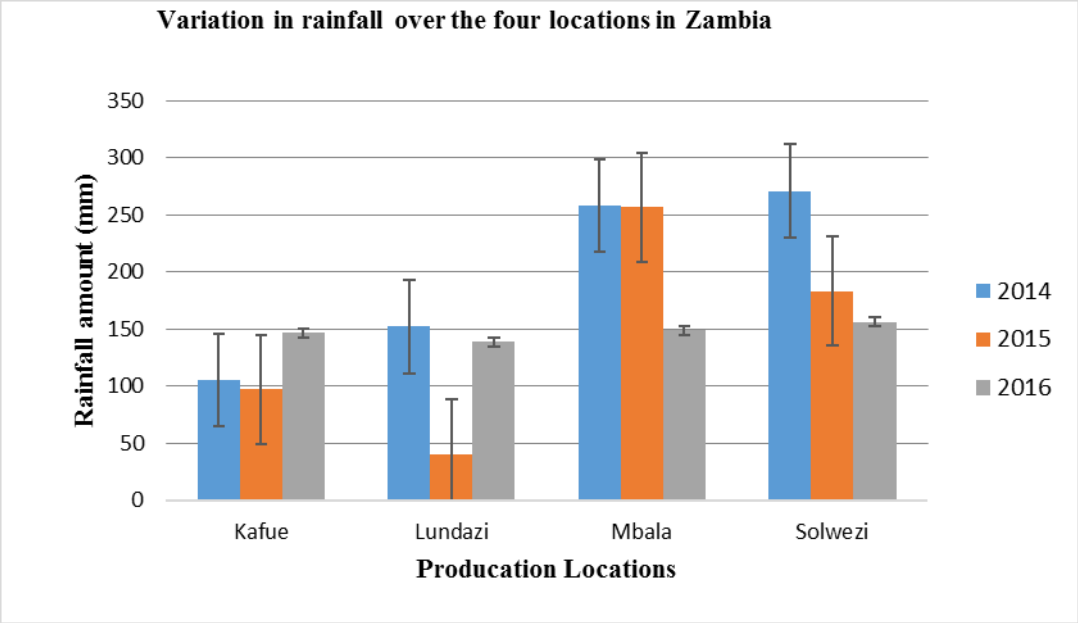


Figure 4.2 Variation in rainfall amounts in Kafue, Lundazi, Mbala and Solwezi across the three years. The mean for January, February, and March are considered for each year. **Source:** Primary data from the Zambian Meterological Department.

As shown in Figure 4.2, high rainfall was received in Mbala (663.88mm) and Solwezi (610.83mm), while the lowest was in Lundazi (331.28) as a total for all the years. The highest rainfall was received during 2014 (786.23mm), and the the lowest was during 2015 (578.18mm) for all the locations. Based on the error bars, there were a lot of variations in rainfall amounts among these locations, and years.

The minimum and maximum temperature did not vary much across the years and locations (Figure 4.3). The minimum temperature ranged from 16.3^0C in Mbala to 17.97^0C in Lundazi,

while the maximum temperature ranged from 26.93^{0}C in Mbala to 29.33^{0}C in Lundazi. There is no significant difference ($p \leq 0.05$) between these values across locations and years. Relative humidity in Solwezi (Figure 4.4) showed that there is a lot of variation in its percentage across the years, and it tends to follow a similar trend to rainfall.

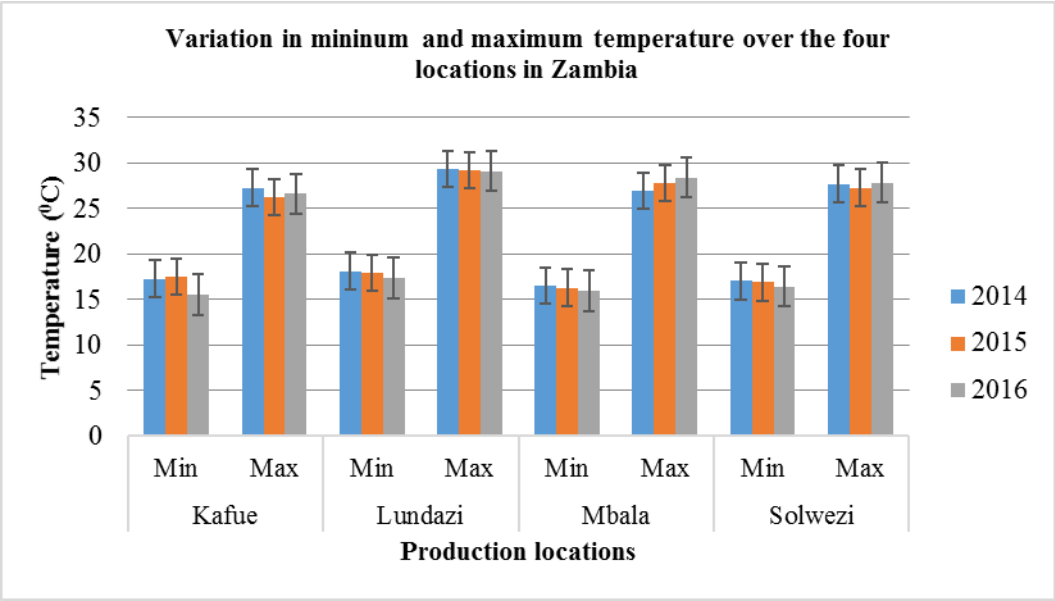


Figure 4.3 Variation in minimum and maximum temperature in Kafue, Lundazi, Mbala and Solwezi across the three yeas. The mean of the mean for January, February, and March were considered for each year. **Source:** Primary data from the Zambian Meterological Department.

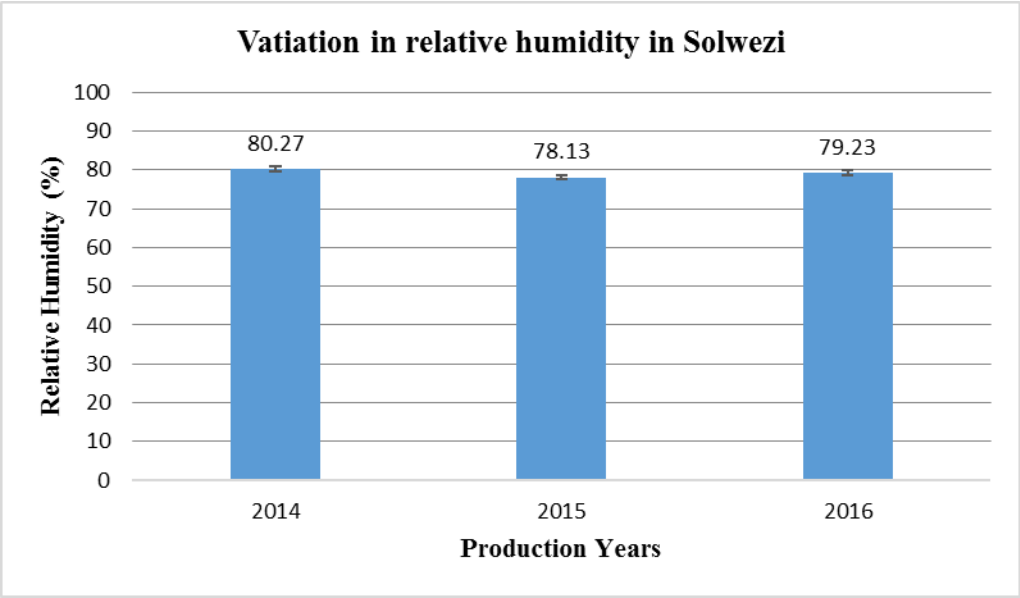


Figure 4.4 Variation in relative humidity in Solwezi across the three yeas. The mean for January, February, and March are considered for each year. **Source:** Primary data from the Zambian Meterological Department.

4.2.4.3 Edaphic factors variation

Semi-structured interviews were used to obtain information on soil types, soil acidity or alkalinity (pH), soil toxicity, soil organic matter, and general soil nutrient levels used for bean cultivation. For example, Professor Obed Isaac Lungu of UNZA said that:

> *The soils of Zambia follow a similar pattern with the three known Agro-ecological zones, and that the four locations of Lusaka (Kafue), Lundazi, Mbala, and Solwezi all fall in Agro-ecologies II and III only that are characterised by highly weathered soils, mainly of Ultisols, Alfisols, and Oxisols soil types, that have low soil organic matter (SOM), low soil pH, known Aluminium toxicity, low cation exchange capacity CEC), and low phosphorous* (Interview, UNZA)

An interview with Mr Raby Banda of Mt Meru Soils Department of ZARI focused on soil acidity (pH) and other limitations is presented in Table 4.1 below. He provided results of soil pH from different surveys across Zambia, and some relevant literature that were very useful for this study. Mr Banda specifically mentioned that:

> *As you move from South to North of Zambia, soil acidity (pH) decreases so greatly, that is, becomes more acidic and the amount of rainfall increases, whereas as you move from West to East, soil texture changes from sandy to clay soil, through clay loam in between and the*

population density becomes denser (Interview, ZARI). *He also provided the values for soil pH by the different locations presented in table 4.5.*

Based on the use of this secondary literature, it became clear that, Agro-ecologies (Figure 4.5), soil types and other limitations (Figure 4.6 and Table 4.5) vary considerable within Zambia. It also became clear that the four study locations did not fall under the two Agro-ecologies II and III only as was mentioned by Professor Obed Isaac Lungu. Kafue and Lundazi has a small section of Agro-ecology I (Figure 4.5), while different soil types are found in each study locations (Figure 4.6). The dominant soil type(s) by districts are: Acrisols for Mbala, Ferralisols for Solwezi, Leptisols for Kafue, and Acrisols, Lixisols, and Solonet for Lundazi (Table 4.5), making Lundazi the most varying in terms of dominant soil types. It also became clear that, the low CEC that Professor Lungu mentioned above was mainly a result of low Ca^{2+} and Mg^{2+} ions.

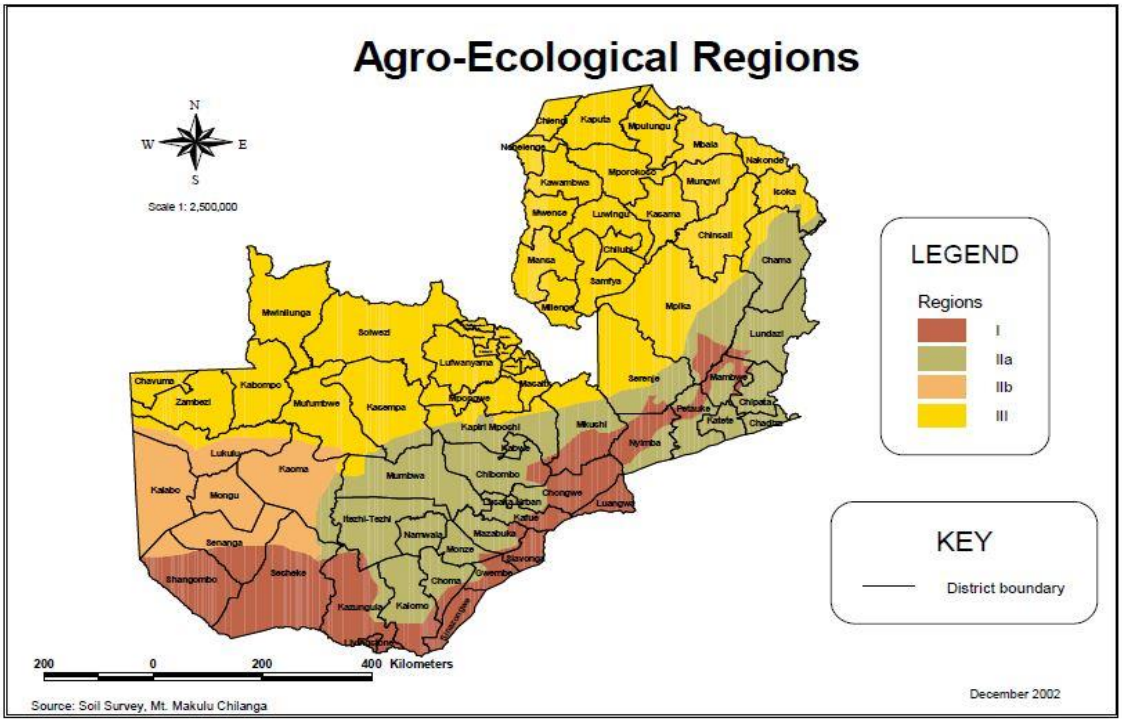


Figure 4.5 Zambian Agro-ecological regions. Adapted from Mwale et al., 2007.

Table 4.5 Details of districts, agro-ecology, soil types, soil acidity (pH), soil limitations, and management options. Modified from Mwale *et al*., 2007 and an interview with [redacted]

Districts	**Agro-ecology**	**Common soil type/ group classification**	**Soil pH[a]**	**Soil limitations**	**Management options**
Mbala	III	Acrisols*, Leptisols, Planosols, Lixisols, Gleysols	3.0 – 4.5	-Soil Acidity -Aluminium toxicity -Low base retention (Ca^{2+} and Mg^{2+}) -Low soil organic matter -Soil compaction and erosion	-Application of lime -Fertilizer application (N,P, K, S, Ca, and Zn) -Soil Organic matter maintenance -Agricultural diversification
Solwezi	III	Ferralsols*, Lixisols, Gleysols, Histosols, Acrisols	3.4 – 4.8	-Soil Acidity -Aluminium toxicity -Low soil fertility -Leaching of nutrients -Compaction	- Application of lime -Fertilizer application (N,P, K, S, Ca, and Zn) -Soil Organic matter maintenance -Agricultural diversification
Kasama	III	Acrisols*, Fluvisols, Gleysols	3.2 – 4.5	-Soil Acidity -Aluminium toxicity -Low base retention (Ca^{2+} and Mg^{2+}) -Low soil organic matter -Soil compaction and erosion	- Application of lime -Application of lime Fertilizer application (N,P, K, S, Ca, and Zn) -Soil Organic matter maintenance -Agricultural diversification
Mpika	IIa and III	Acrisols*, Fluvisols, Arenosols, Gleysols, Leptosols, Lixisols	4.0 – 4.5	-Soil Acidity -Aluminium toxicity -Low base retention (Ca^{2+} and Mg^{2+}) -Low soil organic matter -Soil compaction and erosion	-Application of lime -Fertilizer application (N,P, K, S, Ca, and Zn) -Soil Organic matter maintenance -Agricultural diversification
Kafue	I and IIa	Leptisols*, Acrisols,	6.4 – 6.9	High Calcium accumulation -Shallow, gravely, and stony	-Range land management for non-arable areas -Application of lime

		Lixisols, Luvisols		soils -Soil erosion -Poor drainage	-Fertilizer application (N,P, K) -Soil Organic matter maintenance -Agricultural diversification
Lundazi	I and IIa	Acrisols*, Lixisols*, Solonetz*, Leptisols, Alisols	4.8 – 6.2	-Soil Acidity -Aluminium toxicity -Low base retention (Ca^{2+} and $Mg^{2+)}$ -Soil compaction (dry) -Soil salinity -Sticky/slippery (wet)	-Application of lime -Fertilizer application (N,P, K, S, Ca, and Zn) -Soil Organic matter maintenance -Agricultural diversification -Irrigation - Tolerant crops diversification

*Dominant sil type(s) /group(s) classification by district. Adapted from Mwale *et al*., 2007, [a]Primary Research data from Mt Mweru Soil Department, ZARI.

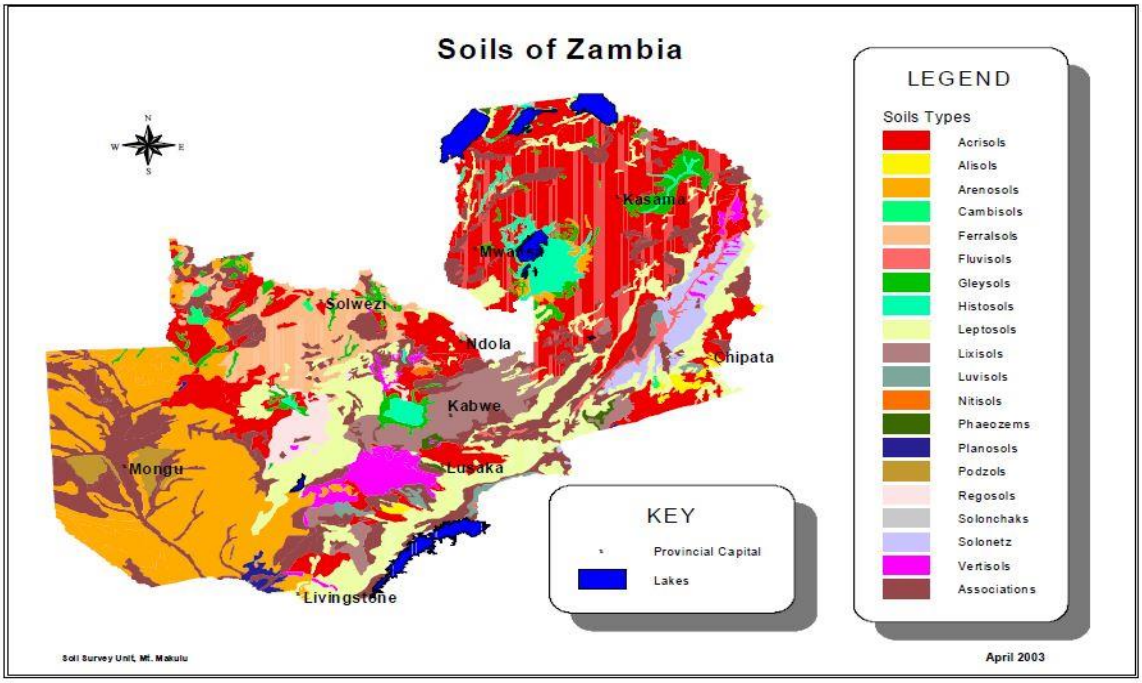


Figure 4.6 Zambian major soil types by regions. Adapted from Mwale et al., 2007.

4.2.5 Roles of private institutions in maintaining common bean diversity

The roles of private companies were considered mainly from the seed company (Seed Co) and an NGO (Self Help Africa) perspectives, on how their interventions could shape up crop diversity of different crops, including the common bean. A group discussion with the Seed Co indicated that they deal mainly in hybrids for Maize and Groundnuts; and that common bean is not their priority crop. The reason they cited was that the beans have a very low rate of return as farmers typically save seeds and only buy infrequently. On the other hand, a group discussion with Self Help Africa staff members indicated that they consider diversity of crop varieties as nutritional safeguards. In doing so, they are working in partnership with the ZARI in selected areas of Zambia to promote bean production and marketing. They are directly involved in the training of these farmers in best agronomic farming practices (through their agricultural advisor staff) to improve their production, particularly yields, and the different nutritional product that can be got from these common bean landraces (through their nutritional advisor staff) to improve utilisation. Therefore, they are working together with the ZARI to promote the use of bean mixtures within these landraces of common-bean, and are pushing for the registration of landraces so that the seeds from them can be marketed nationally and regionally. Besides common beans, they are also involved in Maize, Groundnuts, Sorghum, and others of different varieties to promote food security and livelihood development within their areas of operations in Zambia.

4.3 Discussion

The maintenance of the bean landraces was done participatorily between the ZARI breeders and the selected bean farmer groups in Kafue, Lundazi, Mpika, and Kasama research Institute, yet these landraces were collected from Kafue, Lundazi, Solwezi and Mbala districts, which introduce the two aspects of genotype by environment (GxE) interactions and different farming practices amongst these farmer groups. This could be one factor that can account the differences in genetic diversity that might be observed amongst these landraces as Polegri and Negri. (2009) and Negri *et al*. (2010) had reported that landraces are characterised by a specific adaptation to the environmental conditions of their area of cultivation. This means that, collecting the landraces from different areas, and maintaining them in other areas would directly subject them to GxE interactions that could affect seed composition and types (colour, shape and size) over a long period of time, say over 100 years as reported by Beebe *et al*. (1997). Again, involving different farmer groups in producing and maintaining these landraces while bulking the harvested products would increase human errors in the production chain that

can affect genetic diversity differently. Different farmer groups employ different farming practices, and perhaps customs as dictated by their soil conditions, and their access to resources such as spraying, fertilizer application, staking of climbing beans amongst others. This too can introduce variation in the crops that are being grown, and affecting their level of diversity.

Group discussions with bean farmers and sellers in Kafue and Lundazi pointed out a lot of characters that guide their selection criterion for new variety adoption and subsequent marketing. Zambian breeders should aim at achieving some of these characters in their new varieties to be released and promoted. The breeders and farmers can also work together through the participatory plant breeding, and both should share what exactly they need during this partnership. As Dr Kennedy Muimui pointed out, farmers have associated some of these characters in successful crop varieties, which can easily be adopted if such a variety is released. Asfaw *et al*. (2012) through their participatory approach in common bean breeding for drought tolerance in Southern Ethiopia showed that farmers had their set of characters that they use as selection criteria for drought tolerant bean varieties, chief among these was pod length and the overall yield. Additionally, Assefa *et al*. (2005) had noted a remarkable difference among the different farmers categorised as commercial, resource poor, male and female regarding the importance of these characters, although overall it was closely associated with yields and yield components. This is a component that was not investigated during this study although aspects came out indirectly, such as female farmers being more concerned with the culinary qualities compared to men, and that these characters also would differ between breeders and farmers.

The common bean breeders in ZARI and UNZA are actively using some of these common bean landraces, such as Lusaka yellow, Solwezi and Kabulangeti in their breeding programmes for different objectives. However, there is a uniformity in using Lusaka yellow in their breeding programmes for the two institutions of ZARI and UNZA. The choice for Lusaka yellow could be attributed to these main reasons: ready market for it across Zambia, stable yields across different Agro-ecological zones as presented in Table 4.4, and lack of well characterised available materials for use in bean breeding programmes. UNZA breeders use Solwezi in their breeding programme, and this could be associated with high yield for this landrace in Kafue (Table 4.4), and the appealing seed types within its sub-populations. All breeders are addressing the production constraint of diseases, pests, low nutrients levels of

these bean varieties, among others, pointing out that the challenges of bean production cuts across the Eastern and Southern African regions making material exchange within these locations a viable option to address these challenges. However, as Dr Kelvin pointed out, it very important to start with the single seed/plant when using these landraces in the breeding programmes to allow for reproducibility in the future work.

Variation in farming systems, climatic and edaphic factors were observed across the study areas. The farming systems changed from the reported 5 systems of shifting cultivation, semi-permanent hoe system, semi-permanent hoe and ox plough system, semi commercial cultivation, and commercial systems (Chomba, 2004) to the three systems of small, medium and large farming systems. This change in the farming systems could be attributed to the increase in population density of 4 people per square kilometre in 1955 to 24 people per square kilometre in 2017, which is further predicted to increase to 55 people per square kilometre in 2050 (Worldometeters Statistics, 2017). This same increase in the population density could be the reason for reduced periods of fallow in the crop rotation programmes reported by Kwesiga *et al*. (2003).

Climatic data particularly rainfall and relative humidity varied significantly between the study sites with temperature being less variable. These results differ from the variation in temperature and rainfall was previously reported by Phiri *et al.* (2013) across Zambia. The year 2015 of the study period stood out as the one with the lowest rainfall amounts and the highest temperature, with Lundazi being the most affected site. Relative humidity didn't have a complete data set for all the locations of this study, although results from Solwezi showed a similar trend to rainfall. This implies that, either there is shortage of man power or available funds allocated to collect accurate weather information that needs to be addressed by the government of Zambia if the Zambians are to benefit from the precision farming that requires accurate weather information. Temperature (both minimum and maximum) did not significantly differ across all locations, and any level of diversity in these locations will not be associated to temperature.

The soils of Zambia varied greatly in terms of soil types and pH, alongside the general nutrient levels of the soils. Kafue, Mbala, and Solwezi had one major dominant soil type, whereas Lundazi had three dominant soils types, with varying levels of overlaps between the locations. Following the semi-structured interview at ZARI and UNZA it stood out that, low soil pH,

CEC, and aluminium toxicity is the major cause of yield limitations in major part of Northern Zambia (Agro-ecological zone III) and while in Kafue it is the high calcium accumulation, and shallow soils. Chabala *et al*. (2014) mapped the spatial variability of soil acidity in Chongwe-Rufunsa area in Lusaka Province, South Eastern Zambia, and the minimum soil pH was 4.02 while the maximum pH value was 5.56. This result contradicts the pH values from the semi-structured interview with Mr Raby Banda, and it indicates that there is a lot of variation in the Zambian soils, with more studies required to explore some of these variations. Lungu, and Dynoodt (2008) reported that soil acidification and decreased CEC mainly Ca^{2+} and Mg^{2+} results from long-term use of urea as fertilizer. From these observations, it can be seen that different locations will employ different options to manage soils and water differently making an area that is important for investigation to be how this diversity in soil conditions affects crop diversity, including the common bean.

In understanding the potential of seed companies and NGOs in promoting the genetic diversity of major crops, the challenges that was reported by (Soniia, 2004; Grossman *et al*., 1991; Croomwell and Wiggins, 1993) regarding the marketing of seeds of self-pollinating crops, vegetatively propagated crops, and crops with limited demand by the seed companies were confirmed. Common bean being a self-pollinating crop, it can therefore be concluded that the low rate of return associated with the purchase of its seeds as presented by the seed company could be associated to this fact as farmers do not need to come to buy new seeds from time to time. However, what is crucial to note here is that the goal of the NGO or private company will affect the level of genetic diversity of these major crops in contrasting ways. For instance, Seed company would be interested in few crops mainly hybrids that give farmers higher yields and they benefit from high rate of returns associated with the sale of their seeds, whereas the NGOs would promote a variety of different crops to broaden nutritional quality and livelihood development as a whole, making NGOs better diversity promoters than seed companies. The National Research Institutes (NRIs) and the National Breeding Programmes (NBP), International Centre for Tropical Agricultural (CIAT), and Pan Africa Beans Research Alliance (PABRA) will always work with the different players within the bean value chain with the objectives of protecting the genetic diversity and consumers from the climatic and edaphic challenges by breeding varieties that suit their growing conditions and with enhanced nutritional contents in a participatory manner.

This chapter has set a baseline for the subsequent investigations by pointing that the study districts of Zambia differ significantly in terms of climatic factors, edaphic factors and farming systems. It further showed that the common bean farmers and sellers have their preferences that slightly differ between locations as well as variation in the agronomic practices. The variation in rainfall and relative humidity over the locations and three seasons (2014 to 2016) was significantly important. The role of breeders, private companies and NGOs in maintaining genetic diversity of crops were also explored. It is therefore important to relate how these variation in biophysical factors, farming systems, bean breeding programmes amongst others significantly associate with the level of genetic diversity over the growing seasons and across all the study locations in the next chapters of this thesis based on molecular, agro-morphological and a combination of them.

Chapter 5

Assessment of Genetic Diversity and Population Structure of the Zambian common bean Landraces using Microsatellite Markers

5.1 Introduction

In Zambia, grain legume production is increasing and common bean ranks second after groundnuts, making up about 32.1% of the total area under food legume crops (Hamazakaza *et al*., 2014). Zambia's agricultural policies have been adjusted and refocused to emphasise crop diversification and the inclusion of low-input crops such as food legumes that has enabled a shift from maize dominated production to maize-legumes intercrops, which are more appropriate for resource-poor small-scale farmers and has led to increasing bean production (Siame *et al*., 1998; Hamazakaza *et al*., 2014). Common bean production in Zambia is affected by a number of biotic and abiotic stresses, and other factors, including, insect infestation, disease, climate related constraints, widespread use of low yielding varieties and lack of trusted seed companies (Katungi *et al*., 2009; Muimui *et al*., 2011; Muthomi *et al*., 2011; Hamazakaza *et al*., 2014). The combined effects of these constraints have led to both improved varieties and landraces dominating production in Zambia, with the bean farmers practicing seed admixture, in which improved varieties are planted together with the landraces (Hamazakaza *et al*., 2014), although in some locations landraces dominate. As a result, consumers normally accept a wide range of seed colours in the admixture (Blair *et al*., 2010), except in the urban markets like in Lusaka, where preference for a single seed colour bean seeds occurs. Landraces represent a genetically diverse crop materials that are traditionally maintained and grown by farmers (Soleri and Cleveland, 2004). Each landrace has a distinct but variable population, usually has a common name, lacks 'formal' crop improvement, but is characterised by adaptation to the environmental conditions of the area of cultivation (Negri *et al*., 2009; Galluzzi *et al*., 2010).

Due to their local adaptation, genetic diversity and possession of multiple traits, common bean landraces have been widely used to develop and map SSRs markers to characterise and map anthracnose resistance, derive a major QTL for common bacterial blight resistance, and have shown a useful potential for the improvement of common bean as a crop for the future (Miklas *et al*., 2003; Munoz-Perea *et al*., 2006; Gonçalves-Vidigal *et al*., 2009; Gonçalves-Vidigal *et al*., 2011; Schmutz *et al*., 2014; Sousa *et al*., 2015). Gonçalves-Vidigal *et al*. (2009) observed that there is a need to characterise more anthracnose resistance genes for common bean due to resistance break down to some of the races of the anthracnose disease causative organism

(*Colletotrichum lindemuthianum*). Therefore, this confirms that common bean landraces can be an important source of materials to bean breeders, farmers and conservationists. Breeders will continue to use common bean landraces as a genetic resource with which to improve commercial varieties, farmers would benefit from registering their landraces in order to promote quality control for their maintenance and production, and conservationists will maintain genetic resources for future use (Ceccarelli *et al*., 2000; Raggi *et al*., 2013). To enable landraces to be used and conserved efficiently, their genetic diversity has to be first characterised and determined, thereby facilitating identification for various applications including as parental lines in breeding programme, and aiding seed registration. This assessment of genetic diversity is a major challenge for African landraces, including those from Zambia, due to the fragmentation and isolation of the locations where they are grown, that makes their collection very difficult, in addition to the costs involved.

Simple sequence repeat (SSR) or microsatellite markers have been the most widely used marker system for diversity evaluation in common bean. SSR markers are very reproducible and polymorphic, abundant/wide distribution in the genome, co-dominant, are highly multiallelic, can be genotyped on semi-automated sequencers using multiplex assays, and prove effective in distinguishing the genepool and race structure of common bean (Buso *et al*., 2006; Diaz and Blair, 2006, Blair *et al*., 2009b; Okii *et al*., 2014b). Blair *et al*. (2013a) noted that SSR markers are an efficient multiallelic marker system for diversity analysis compared to other single locus markers, such as single nucleotide polymorphisms (SNPs), since they provide over 10 times the information. The usefulness of SSR markers for this purpose is confirmed by their ability to distinguish between inter- and intra-genepool introgression, including domesticated through wild to the weedy species (Blair *et al*., 2003 and 2009a; Zizumbo-Villarreal *et al*., 2005).

The main goal of this chapter is to determine the genetic diversity within and between the four major Zambian common bean landraces, which show varying levels of admixture, using a well characterised set of 20 SSR markers. Zambia is bordered by four of the top ten producers of common bean in Sub Saharan Africa (SSA), Democratic Republic of Congo (DR Congo), Tanzania, Malawi and Angola, where landraces are also dominant in common bean production. Due to the porous nature of their borders, common bean materials of unknown origin probably find themselves in Zambia, thereby contributing to and altering over time the genetic diversity of Zambian landraces. The specific objectives of this study were: to

determine, the genetic diversity and population structure between and within these landraces, the proportion of the individuals within a landrace falling into each of the genepool, as these are importance component of these landraces to the bean breeding programme in Zambia.

5.2 Results

5.2.1 Screening of SSR markers for their polymorphism and genepool association of the landraces

The usefulness and level of polymorphism of the different markers were assessed, from which 28 of 50 SSR markers were selected for the subsequent entire study (Annex 2). The screening phase also led to the clustering of the different individuals into two distinct clusters representing Mesoamerican and Andean genepools as confirmed by the reference genotypes (Figure 5.1) with very high bootstrap values supporting this clustering pattern and the genepool association. Lusaka yellow and Lundazi belong Andean genepool, whereas Mbala mixture and Solwezi are predominantly Mesoamerican genepool although they have some individuals under Andean genepool too (Figure 5.1).

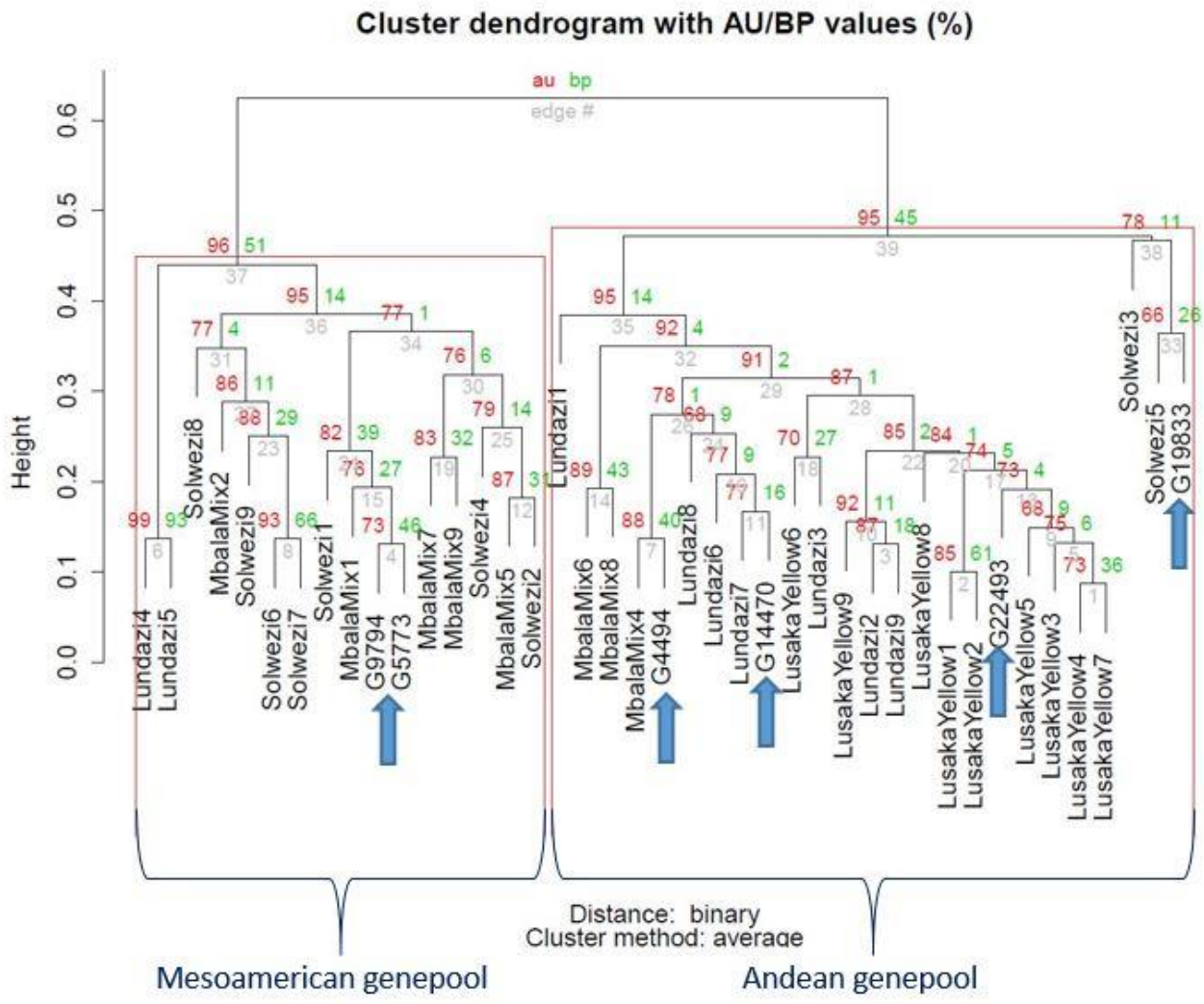


Figure 5.1 Clustering pattern and genepool association of the landrace individuals to the reference genotypes. Thick blue arrow indicates the position of the test genotypes, the red bootstrap values represent approximately unbiased (AU) and green values represent bootstrap probability (BP).

5.2.2 Molecular genetic diversity analysis of the Zambian common bean landraces

Genetic diversity within and between the four Zambian common bean landraces was determined using 20 microsatellite markers (SSRs), all of which detected polymorphic loci, and had proved useful in other studies (Buso *et al.*, 2010; Wang *et al.*, 2012; Blair *et al.*, 2006a; 2009a,b; 2010; Blair and Lorigados, 2016). The SSRs detected a total of 214 alleles, with varying number of alleles per locus, ranging from 2 to 34, with an average of 11 alleles (Table 5.1). The lowest number of alleles recorded was 2 and 3 for Pv-gaat002 and BMd01, and BMd32 and Pv-ag003 respectively, while the highest numbers of alleles were 33 and 34 for BMd28 and C119 respectively. In total, 10 SSR markers produced less than 10 alleles per marker. It important to note here that, the molecular diversity being presented in this chapter is from a pooled populations of 1101 individuals that were run over the three growing seasons of

2014-2016. The parameters that are reported in figures and can be interpreted in different ways for example, the number of alleles shows allelic diversity among the studied populations, and the higher the number the more genetically diverse is the populations.

Similarly, allelic frequency is used to measure the frequency of occurrence for the different alleles. Alleles with higher frequencies occur more frequently in the populations, which implies less diversity within the populations, whereas alleles that occur less frequently shows high diversity in the populations. Therefore, in this study, mean allelic frequency was 0.247 with the frequency of 0.621 and 0.575 for Pv-ag003 and Pv-gaat002 as highest, and 0.081 and 0.086 for BMd28 and C119 as lowest respectively. Shannon information Index (I) which is a measure of genetic diversity ranged from 0.077 (Pv-gaat002) to 1.286 (BMd28) with an average of 0.6049. McClean (2016) showed that, polymorphism information content is a measure of the usefulness of microsatellites for genetic analysis as it allows one to deduce the marker allele that the offspring inherited from the parent. Its value ranges from 0-1.0, with values close to 0 being less useful while figure close to one being more useful in genetic analysis. This current study reports that polymorphism information content (PIC) ranged from 0.379 to 0.919, with an average of 0.753. The most polymorphic markers were C119, BMd28, and BM211, while the lowest were Pv-gaat002 and Pv-ag003. Rate of gene flow (Nm) ranged from 0.0401 to 0.8038 with an average of 0.391. The loci BMd28, C119 and BM211 detected the highest rate of gene flow, while the loci Pv-gaat002 and Pv-ag003 detected the lowest rate of gene flow.

The genetic diversity between and within the Zambian landraces and the CIAT reference lines based on 20 SSR markers were compared (Table 5.2) and the results revealed a high level of difference between the two groups. Generally, all parameters as explained above were highest among the landraces compared to CIAT lines; mean no of alleles (6.4 vs 1.69), mean number of private alleles (0.88 vs 0.02), mean expected heterozygosity (0.517 vs 0.254), Shannon Information Index (1.0342 vs 0.3902), and polymorphism information content (0.8445 vs 0.4386) for the Zambian landraces versus CIAT reference lines, respectively, except for allelic frequencies that were highest in CIAT lines compared to landraces (0.5614 vs 0.1555). Therefore, combining the genetic diversity results from SSR markers and the populations studied, it can be seen that there is a high genetic diversity amongst these Zambian landraces than the CIAT reference lines, and that the diversity differs from population to population.

5.2.3 Population Structure Analysis of the Zambian common bean landraces

In order to answer questions on the population structure, all SSR data collected from the four landraces in three growing seasons (2014, 2015 and 2016) together with those from the CIAT lines were pooled to give 1101 individuals for analysis. The population structure provides a measure of heterogeneity and in this context means identifiable subgroupings within the total populations that share similar characteristics. The STRUCTURE analysis clearly differentiated between the Andean and Mesoamerican genepools (K2) and the different subpopulations therein (K7, 9, and 15) of the Zambian beans (Fig 5.2). While Evanno's test indicated that the most informative number of sub-populations (K) is 7, the Zambian landraces uniquely produced different peaks at K 2, 3, 5, 7, 9, 12, and 15 (Fig 5.3).

A detailed analysis of the populations and/or sub-populations from the STRUCTURE software showed that at K=7, the different subgroups are: A1, with 133 individuals composed of Mbala Mixture and Lusaka yellow; A2, with 183 individuals composed of Lundazi, Solwezi and Mbala Mixture, together with G4494 as the test genotype; A3, with 170 individuals composed of Lusaka Yellow, Lundazi, Mbala mixture and Solwezi, together with the Zambian Commercial line G14470; M1 with 95 individuals composed of Lundazi and Solwezi; M2, with 179 individuals composed of mainly Lundazi; M3 with 173 individuals composed of few Lundazi, Mbala mixture and Solwezi; and M4, with 168 individuals mainly of Lusaka Yellow small seeded beans with a white ring around their helium.

Table 5.1 Genetic diversity indicators for the common bean landraces: Linkage group (LG), Number of alleles (Na), Number of effective alleles (Ne), Shannon information index (I), Observed heterozygosity (Ho), Expected heterozygosity (He), and Polymorphic information content (PIC), Fixation Index (F) and Gene flow (Nm) from the analysis of 20 SSR markers in common beans.

LG	SSR markers	Total Na	Mean Na	Mean Ne	Allele Freg.	I	Ho	He	PIC	F	Nm
1	Pv-ag003	3	2	1	0.621	0.2216	0.0235	0.1460	0.3790	0.8764	0.0887
1	BMd32	3	2	2	0.350	0.5565	0.0000	0.3808	0.6500	1.0000	0.8038
2	BMd07	11	4	2	0.140	0.7528	0.6583	0.4677	0.8600	-0.4387	0.4620
2	PV-BR25	5	2	1	0.295	0.3821	0.0097	0.2398	0.7050	0.9627	0.1800
2	BMd18	11	3	2	0.163	0.5218	0.0398	0.2787	0.8370	0.8638	0.2923
2	BMd03	11	3	2	0.177	0.6095	0.2431	0.3306	0.8230	0.2978	0.2446
2	Pv-gaat002	2	1	1	0.575	0.0776	0.0000	0.0492	0.4250	1.0000	0.0401
3	BMd01	3	2	2	0.334	0.6173	0.0003	0.4275	0.6660	0.9993	1.9600
4	Pv-atgc002	7	3	2	0.242	0.6240	0.0224	0.3765	0.7580	0.9477	0.3400
4	Pv-ctt001	11	3	2	0.225	0.5490	0.0324	0.2643	0.7750	0.9010	0.1319
5	BMd53	5	2	1	0.304	0.4182	0.0225	0.2378	0.6960	0.9184	0.1701
5	BMd28	33	8	3	0.081	1.2860	0.7130	0.6343	0.9190	-0.1314	0.6525
5	Pv-at006	5	3	2	0.270	0.7618	0.5417	0.4288	0.7300	-0.2062	0.3537
6	C119	34	5	2	0.086	0.6317	0.0297	0.2794	0.9140	0.9077	0.1062
6	BM137	13	4	2	0.119	0.7179	0.2428	0.3791	0.8810	0.2315	0.3246
8	BM211	19	5	3	0.096	1.1240	0.8611	0.6122	0.9040	-0.5171	0.7014
9	Pv-tttc001	4	2	1	0.338	0.2773	0.0585	0.1666	0.6620	0.5713	0.1497
9	Pv-at007	7	3	2	0.236	0.6103	0.5495	0.3702	0.7640	-0.4659	0.2602
9	Pv-gat001	13	4	2	0.154	0.6640	0.1402	0.3801	0.8460	0.6866	0.2710
11	BM33	14	4	2	0.134	0.6940	0.0977	0.3792	0.8660	0.6834	0.2795
Grand Total		214	65	37	4.940	12.0972	4.2862	6.8290	15.0600	10.0883	7.8122
Grand Mean		**11**	**3**	**2**	**0.247**	**0.6049**	**0.2143**	**0.3414**	**0.7530**	**0.5044**	**0.3906**

Table 5.2 Number of individuals and Mean allelic parameters by Populations: Number of individuals (N), No. of alleles (Na), No. of effective alleles (Ne), Shannon Information index (I), Expected Heterozygosity (He), Polymorphic information content (PIC), and Percentage of polymorphic loci (% Poly) across different populations studied grouped as Landraces and CIAT Lines analysed by 20 SSR markers

Population	N	Mean Na	Mean Ne	Mean no of private alleles	Allele Frequency	I	He	PIC	% Poly
Landraces									
LusakaYellow	257	6.15	2.36	1.20	0.1630	0.8920	0.4480	0.8370	95%
Lundazi	262	6.65	2.71	1.15	0.1490	1.0773	0.5368	0.8510	95%
MbalaMixture	262	6.70	2.64	0.70	0.1490	1.0582	0.5197	0.8510	100%
Solwezi	262	6.10	2.66	0.45	0.1610	1.1095	0.5646	0.8390	100%
Total	1043	25.60	10.37	3.50	0.6220	4.1370	2.0691	3.3780	390%
Mean	**261**	**6.40**	**2.59**	**0.88**	**0.1555**	**1.0342**	**0.5173**	**0.8445**	**98%**
CIAT Lines									
G9794	8	1.85	1.52	0.00	0.5140	0.4266	0.2684	0.4860	60%
G5773	8	1.55	1.39	0.00	0.5930	0.3582	0.2343	0.4070	50%
G4494	8	2.05	1.79	0.00	0.4630	0.5038	0.3023	0.5370	60%
G19833	8	1.75	1.64	0.15	0.5710	0.4469	0.3004	0.4290	60%
G14470	8	1.70	1.46	0.00	0.5590	0.3781	0.2482	0.4410	50%
G22493	8	1.65	1.40	0.00	0.5760	0.3586	0.2360	0.4240	55%
G4494A	5	1.45	1.34	0.00	0.6210	0.3077	0.2037	0.3790	45%
G4494B	5	1.50	1.42	0.00	0.5940	0.3414	0.2350	0.4060	50%
Total	58	13.50	11.96	0.15	4.4910	3.1214	2.0283	3.5090	430%
Mean	**7**	**1.69**	**1.50**	**0.02**	**0.5614**	**0.3902**	**0.2535**	**0.4386**	**54%**
Overall Total	1101	39.10	22.33	3.65	5.1130	7.2583	4.0974	6.8870	820%
Overall Mean	**134**	**4.04**	**2.04**	**0.45**	**0.3584**	**0.7122**	**0.3854**	**0.6416**	**0.7563**

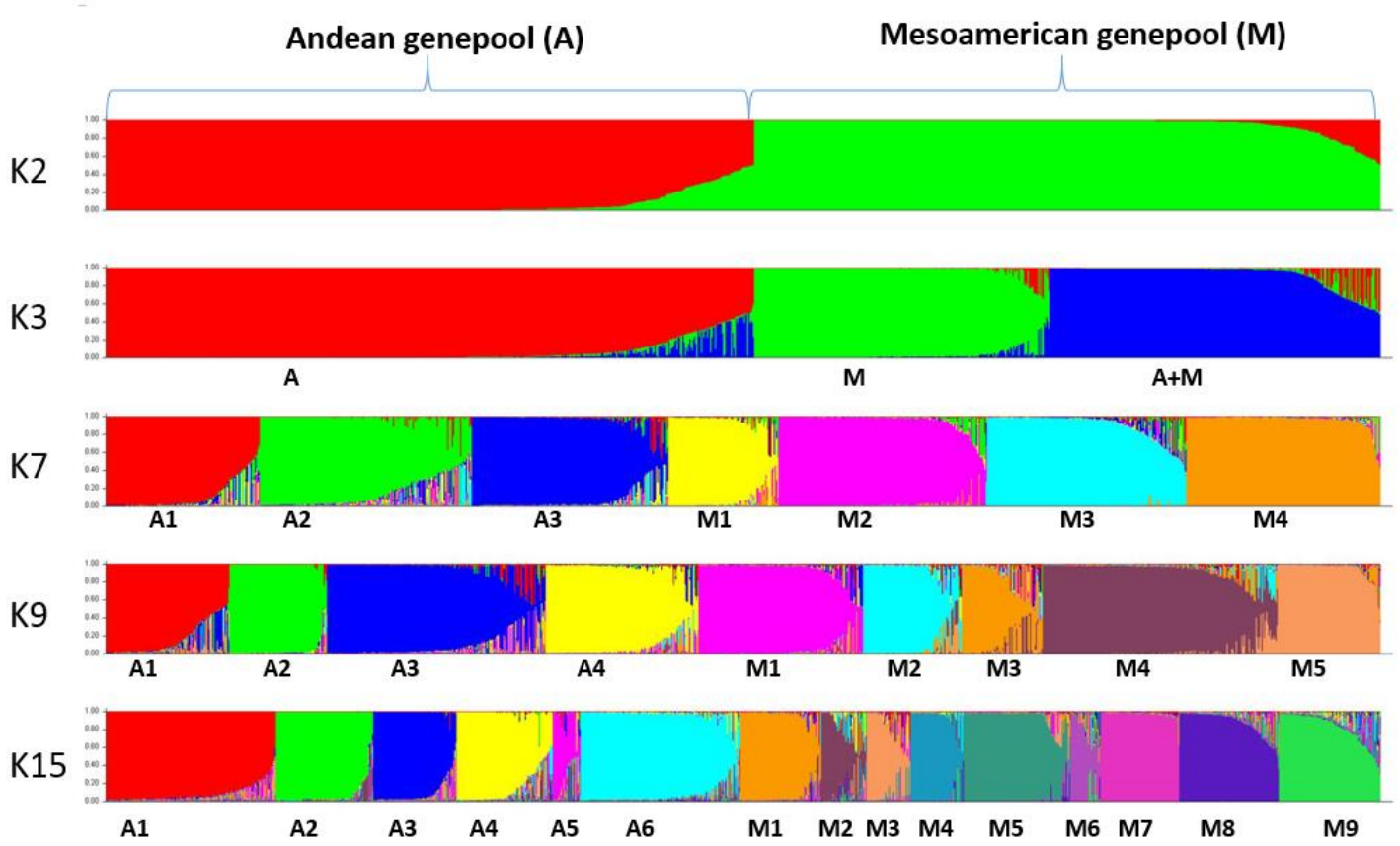


Figure 5.2 Population structure at K2, K3, K7, K9, and K15 for 1101individuals of the Zambian common bean landraces and the CIAT referenced lines. The distinction between Andean and Mesoamerican genepools (K2), Introgression between the two genepools at K3 (A+M), Andean and Mesoamerican subgroups at K7 (Andean 3 sub-populations and Mesoamerica 4 sub-populations), K9 (Andean 4 sub-populations and Mesoamerica 5 sub-populations) and K15 (Andean 6 sub-populations and Mesoamerica 9 sub-populations). The vertical lines separating the individuals in the STRUCTURE bar diagrams, while the different colours of the each bar line represents the genetic composition of that individual.

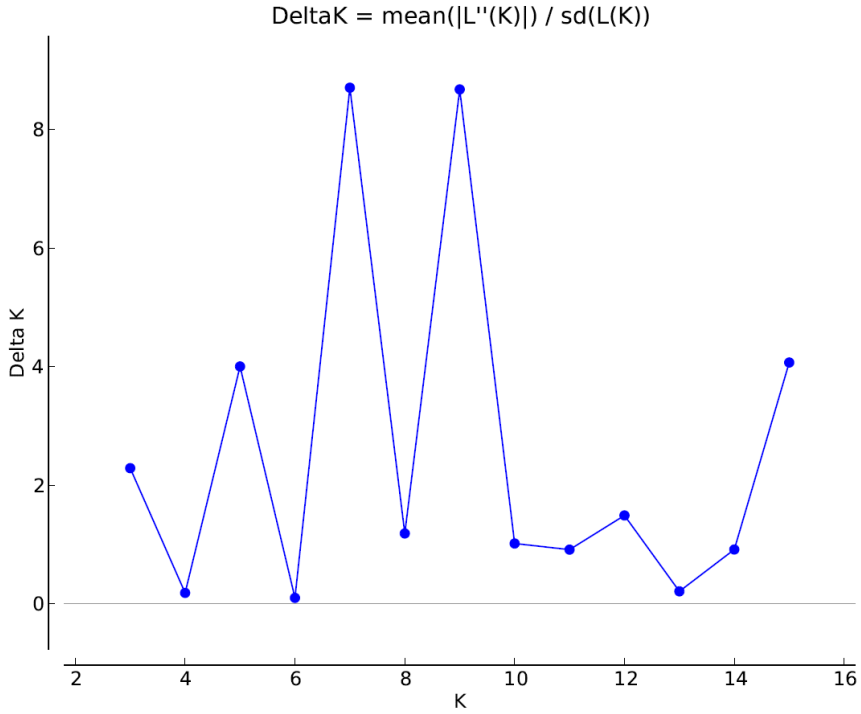


Figure 5.3 Estimation of the optimum number of clusters for the Zambian common bean landraces according to the Evanno's method implemented by Structure Harvester as described by Earl and vonHoldt (2012). The graph displays the DeltaK [mean(IL"(K)I)/SD(L(K))] for each K value, and the highest K values is the optimum number of sub-populations, which in this study is 7, although 9 is also very high.

Principal component analysis (PCoA) produced similar results to the above, clearly distinguishing the two genepools of Andean and Mesoamerican as revealed by the test genotypes. Taking each population genetic diversity averages, Lusaka Yellow and Lundazi belong predominantly to the Andean genepool, while Mbala mixture and Solwezi to the Mesoamerican genepool (Figure 5.4, Left). There was also a very close association between the CIAT line(G22493) a landrace that was collected from Zambia, and the Zambian landrace (Lusaka yellow) used in this study that interestingly share the same seed colour (yellow with black helium ring) and seed shape (oval). Sorting the seed each landrace by seed colour, size and shape, revealed genepools admixture for all landraces as their sub-populations belong to both Andean and Mesoamerican genepools (Figure 5.4, Right). This implies that these local farmers are maintaining germplasm from both centres of origin, which can be very useful in common bean improvement programmes, as focusing in any landrace can mean working with materials from all the two genepools, thus broadening genetic base.

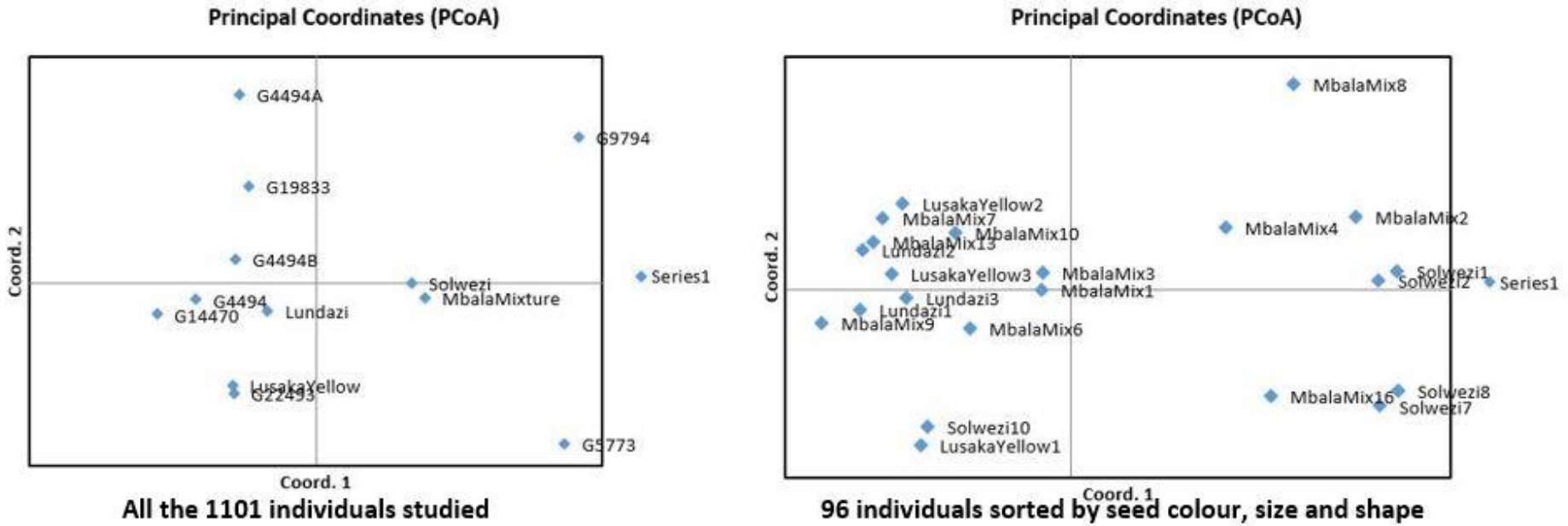


Figure 5.4 Principal coordinates analysis (PCoA) of the populations from the 20 microsatellites diversity based on the Covariance of Nei's unbiased genetic distance. Andean genepool: Lusaka Yellow and Lundazi; and Mesoamerican genepool: Mbala Mixture and Solwezi predominantly. Left is for all the individuals studied, and right is for the sorted seeds that confirmed that some individuals of Solwezi and Mbala Mixture belong to the two genepools. The Zambian Landrace (G22493) and a commercial variety (G14470) from CIAT genebank both belong to the Andean genepool.

5.2.4 Population differentiation

Analysis of Molecular variance (AMOVA) showed that there is a highly significant ($p < 0.001$) variation among the Zambian landraces and/or the reference CIAT lines, and within the individuals of these populations (Fig 5.5) when analysed together. The overall allelic diversity was partitioned into 21 percent among populations, and 59 percent among individuals and 20 percent within individuals ($p < 0.001$). As mentioned above, the value for fixation index (Fst) can be used both to deduce population differentiation and the level of outcrossing at the same time. The Fst values range from 0-1.0. The values close to 0 means a high population differentiation whereas values close to 1 mean that the population is fixed. In this study the population genetic differentiation (Fst) value was 0.210, co-efficient of inbreeding (Fis) 0.746, and rate of gene flow (Nm) 0.940 ($p < 0.001$). These three values mean that there is a high population differentiation, a high proportion of inbreeding and the rate of gene flow is high; hence, there is a moderate outcrossing amongst these landraces. Sorting seeds of each landrace according to size, shape and colour resulted in different partitioning of allelic diversity parameters as 46% among population, 32% among individuals and 22% within individuals based on the same set of SSR markers. The values for Fst 0.461, Fis 0.586, and Nm 0.292 ($p < 0.001$) were equally affected when the seeds were sorted by seed colour, size and shape under each category. Sorting the seed reduces individual admixture among these landraces and within each landrace, therefore increases population fixation, and inbreeding; thus it reduces gene flow.

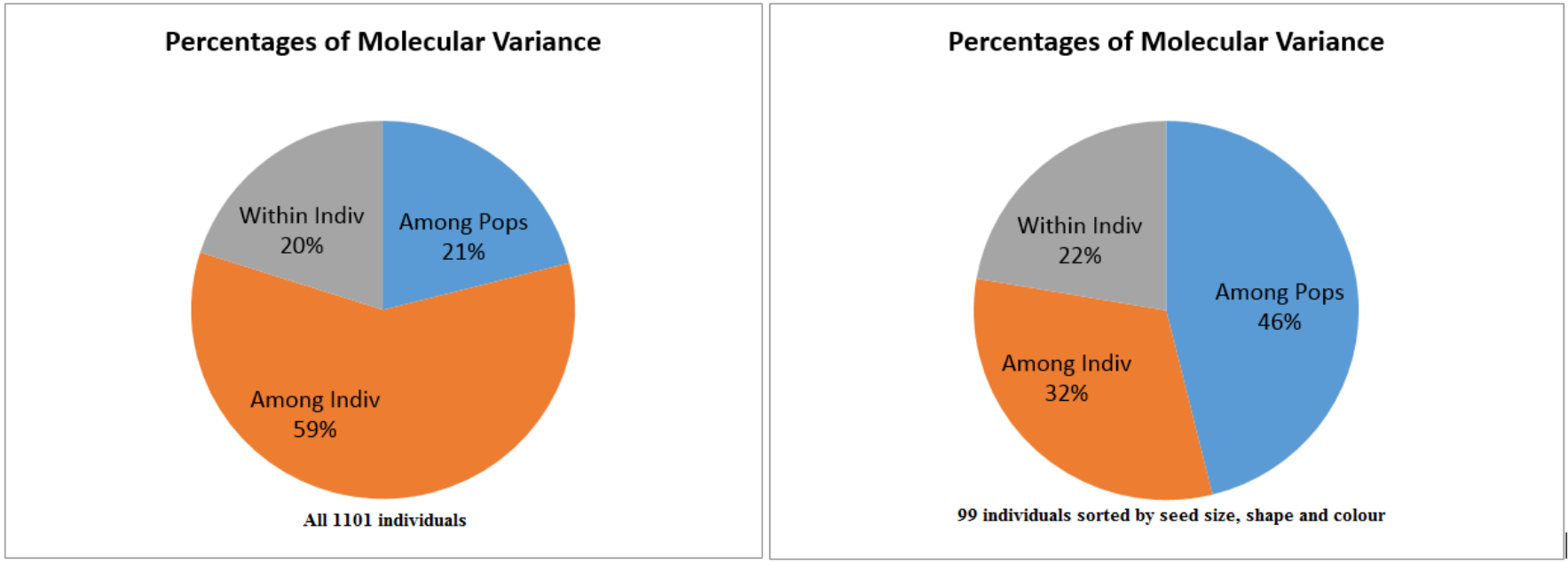


Figure 5.5 Analysis of Molecular Variance (MANOVA) partitioning percentages of observed variation among populations, among individuals and within individuals (Left). Representative samples for each landrace were randomly selected and sorted by size, shape and colour and the results showed an increase in population variance with a decrease in individual variance (Right).

5.3 Discussion

The main objective of this study was to determine the genetic diversity and population structure of the common bean landraces from Zambia. Using CIAT reference lines that were used in previous studies, the study further aimed at assigning these landraces to their respective genepools, which can be achieved using both morphological and molecular markers (Blair *et al*. 2006; Diaz and Blair, 2006; Kwak and Gept, 2009). These approaches reveal that the relative proportions of Andean and Mesoamerican beans vary in different geographical areas (Angioi *et al*., 2011; Belluci *et al*., 2014). For example, in Europe, Andean beans dominate (Angioi *et al*., 2010), in Brazil Mesoamerican beans, in China Mesoamerican beans (Zhang *et al*., 2008), in Cuba Mesoamerican beans (Blair and Lorigados, 2016), and in Africa the proportion of Andean to Mesoamerican bean is approximately 50:50 overall (Asfaw *et al*., 2009; Blair *et al*., 2010 and Okii *et al*., 2014b). Therefore, it is important to study more populations of African origin on a country by country basis. Our study of the 1101 individuals of the four landraces from Zambia using 20 microsatellite markers showed that all the markers were polymorphic, and 214 alleles were detected with an average of 11 alleles per locus. The study further reported a high level of genetic diversity within these materials by looking at the average values for Shannon information index (0.605), and polymorphism information content (0.753).

These results are comparable to those reported by Asfaw *et al*. (2009) on the study of common bean from the East African highlands, and Blair *et al*. (2010) on the study common beans from the Great Lake regions using 30 markers (Rwanda and Democratic Republic of Congo – DRC), in which both studies detected an average of 10 alleles per locus. However, the number of alleles reported in this present study on average is lower compared to that of Okii *et al*. (2014b) who they detected 423 alleles with an average of 19 alleles per locus using 22 markers on the germplasm from Uganda; but is higher compared to that of Burle *et al*. (2010) who detected 460 alleles with an average of 7 alleles per markers on the landraces from Brazil, Wang *et al*. (2012) who detected 48 alleles with an average of 3.69 alleles per locus using a newly developed set of 13 SSR markers, and Scarano *et al*. (2014) who detected 85 alleles with an average of 8.5 alleles using 10 markers from the 25 landraces of the Campania region of Southern Italy. The difference in the values reported in this study and other previous studies can be attributed to genetic background of the studied materials, use of gene-based versus genomic SSR markers, sample size, number of polymorphic markers used, and the gene pool from which the SSR markers were developed compared to the gene pool of the studied materials. Specifically, Blair *et al*. (2009a, b and 2012), in their studies of the CIAT core collection and wild beans, reported higher values for

genomic markers compared to gene-based markers. Similarly, the higher values reported by Okii *et al*. (2014b) could also be attributed to this fact as 18 out of the 22 SSR markers they used were genomic not gene-based markers in nature.

In this present study, equal numbers of gene-based and genomic markers were used to distinguish between members within a population, and between populations and gene pools, respectively. More diversity within the landraces compared to CIAT lines can be attributed to the presence individuals from diverse genepools and to the higher number of individuals studied since previous studies had noted that diversity parameters are directionally proportional to the number of individuals studied in each sub groupings (Blair *et al*., 2010; Burle *et al*., 2010 and 2011; Okii *et al*., 2014b). Overall, the genetic diversity of the Zambian common bean landraces was high considering the average number of alleles, allelic frequency, expected heterozygosity, and polymorphic content information with respect to the CIAT reference line and the earlier studies reported above for the African countries. Several factors have been put forward to explain the high genetic diversity in the common bean such as adaptation to soil types and environmental conditions (photoperiod), cultivation techniques of the small scale farmers, and geographical isolation for the Italian landraces (Scarano *et al*., 2014), original introductions and seed admixture for the East African highlands (Asfaw *et al*., 2009). Additionally, seed mixing and/or admixture was singled out by Blair *et al*. (2010) as important in Central Africa, and farmers seed preferences that lead to high selection pressure was reported in Uganda (Okii *et al*., 2014b). Therefore, this research notes that the presence of the porous borders that surround Zambian territory, and the two coastal routes from the East African Indian Ocean and the Western Angolan South Atlantic Ocean as an optimum conditions for seed mixing from geographically diverse backgrounds into Zambia. Further still, the two distinct agro-ecologies of Zambia's locations where the study materials came from could have promoted the selection and development of different common bean sub-populations with different overlaps amongst them, can provide alternative explanations for this observed high genetic diversity within these Zambian common bean landraces.

Results from the population STRUCTURE and principal component analysis (PCA) supported one another and distinguished the Zambian common bean landraces into the two genepools. Considering population averages, Lusaka Yellow and Lundazi beans are predominantly Andean, while Mbala mixture and Solwezi beans are predominantly Mesoamerican, as confirmed by the position of the test genotypes into their two known gene pools (Calima – G4494 and G19833

being Andean and ICA Pijao – G5773 and G9794 Mesoamerican). This was not surprising as most studies (Burle *et al*., 2011; Mercati *et al*., 2013; Raggi *et al*., 2013; Zhai *et al*., 2014) using SSR and EST-SSR markers clearly separated the Andean beans from Mesoamerican beans. However, the detection of sub-populations within each genepool using STRUCTURE analysis within the Zambian landraces produced up to a maximum of K15 that was unexpected, and reflects a very high number of sup-populations within these landraces.

A detail analysis of these sub-populations with Evanno's methods using Delta K produced peaks at different K values of 2, 3, 5, 7, 9, and 15. K7 was the K value with the maximum peak suggesting seven sub-populations among these landraces with three sub-populations (A1, A2, and A3) under Andean genepool, with four sub-populations (M1, M2, M3, and M4) under Mesoamerican gene pool. The population structure described above in this current study agrees with the finding of Asfaw *et al.* (2009) who detected seven sub-populations within the East African highland beans from Kenya and Ethiopia, and further agrees with Blair and Lorigados (2016) who detected peaks at K5 and K7 with the common beans from Cuba. However, this current study contrasts with the population structure reported by Okii *et al*. (2014b) on Ugandan germplasm of common bean and Scarano *et al*. (2014) on the common bean from the Campania region of Southern Italy, where both detected sub-populations at K3. However, these differences could be attributed to differences in the populations studied and SSR markers used, as alluded on earlier.

A detailed look at K7 individuals reveal that within the Andean gene pool, A1 consisted of 133 individuals mostly from the Mbala mixture and Lusaka yellow. They represent a medium size yellow colour bean with dark helium and oval seed shape. The CIAT referenced Zambian landrace also falls within this group. A2 consisted of 183 individuals drawn from Lundazi, Mbala mixture and Solwezi, mainly of medium size mottled seeds. The CIAT reference line Calima (G4494) fell within this sub-population. A3 had 170 individuals drawn from all the four landraces with mixed colours, and the Zambian commercial line also fell within this sub-population. It is most probable that it is in this A3 sub-populations within the Andean gene pool at K7 where more new sub-populations (A4, A5, and A6 subpopulations) appeared at higher K values of K9 and K15, as it had a composition of individuals from all the four landraces. Within the Mesoamerican genepool sub-populations, M1 had 95 individuals of mainly Lundazi and Solwezi with small red beans, M2 had 179 individuals of mainly Lundazi with medium-dark brown seed colour and kidney shape, M3 had 173 individuals of mainly Mbala mixture and

Solwezi of light-pink seed colour with brown/red stripes, and M4 that had 168 individuals mainly of Lusaka yellow with small yellow seeds with white helium. It is important to note here that there was a lot of variation in seed colour within each sub-population.

The unexpected feature of the Zambian landrace population structure that detected of sub-populations up to K15, showed a high level that has not, to my knowledge, been reported previously. The nearest to this high K values was up to K10 for the common bean landraces of Brazil (Burle *et al*., 2011). The K15 finding reported here indicates a high population diversity and a well structured populatiuins within Zambian common bean germplasm. This degree of population structure can be attributed to the high level of gene flow (Nm = 0.39) detected from multiple possible sources: neighbouring countries; original broad introductions into Zambia from CIAT (based at Mbala); selection pressure from modern participatory breeding programmes and/or natural hybridization in the farmers' fields; several domestication centres (Andean and Mesoamerica) for common beans; deliberate seed mixing by farmers; and local market preferences that accept seed mixtures. It is also probable that these insights are due to the large sample size used here (1101 individuals) and the wide extent of bean cultivation environment in Zambia from which these materials came from. Limited gene flow and restricted sampling that have been reported to reduce diversity in secondary compared to primary centres (Blair *et al*., 2009a, b), was not an issue in this current study as were have reported diversity indices from the 1101 individuals from the four mixed landraces of common beans with an average gene flow of 0.392.

The PCoA distinguished the Andean and Mesoamerican gene pools with the first components explaining 84% of the variation between the two groups. Both Structure, and PCA results confirm that the Mesoamerican predominates (56%) compared to the Andean (44%) in the landraces from Zambia. This result is similar to that reported by Blair *et al*. (2010) for Rwanda (58% Mesoamerican and 37% Andean, and the rest being due to introgression), although it differed from those reported for Uganda (50:50), while the Andean was more common than the Mesoamerican genepool in Kenya, Ethiopia, Malawi and Tanzania (Wortmann *et al*., 1998; Asfaw *et al*., 2009; Okii *et al*., 2014b). The preference for Mesoamerican gene pool over Andean in East Africa was attributed to many factors such as market preference for small seeded beans, drought tolerance, resistance to root rot disease, input of germplasm from breeding programs, and stable yields under varied environmental conditions, amongst others (CIAT 1989, Blair *et al*., 2010; Okii *et al*., 2014b).

The analysis of molecular variance (AMOVA) results partitioned the overall variation within the 1101 individuals as 21 percent among populations, and 59 percent among individuals and 20 percent within individuals ($p < 0.001$), whereas when the seeds were sorted by colour, size and shape the variations were partitioned as 46 percent among population, 32 percent among individuals and 22 percent within individuals ($p < 0.001$). These AMMOVA results show that there is less variation among populations than within population due to the fact that individuals within the different populations are overlapping, therefore seed admixture is the reason behind this high genetic variation and population differentiation as sorting the seeds by size, shape and colour reduces the various components of population differentiation and thus increases the variation among populations. McDermott and McDonald (1993) observed that the shared presence of an individual or more between populations is sufficient to prevent different selectively neutral alleles from becoming nearly fixed in different populations, supporting seed admixture as the main reason for this modest population differentiation in this current study. This concurs with studies from the East African highlands of Kenya and Ethiopia (Asfaw *et al*., 2009) and the Great Lake regions (Rwanda and DRC) that found seed mixing and/or admixture as the cause of high genetic diversity for these regions (Blair *et al*., 2010). In Zambia, Hamazakaza *et al*. (2014) showed that landraces are grown together with the commercial varieties in the same farmers' fields, and in Uganda this admixture of landraces and commercial varieties in the same plot was used to manage insect pests (Ssekandi *et al*., 2016). Therefore, it was not surprising to see individuals from mostly Mbala mixture and Solwezi clustering at the midpoint of the PCA, implying that some of those individuals are commercial varieties within the landraces kept by farmers in the same seed lot. In addition to seed admixture, Linhart and Grant (1996) reported toxic soils, fertilizers, mowing and grazing, soil moisture, temperature, light intensity, pollinating vectors, parasitism, gene flow, and natural dynamics as other factors that affect population differentiation in crop species.

The high genetic diversity measured in the common bean landraces implies that there is a major potential resource in the hands of the African farmers that can easily be exploited for bean improvement and that requires conservation for future use. Looking at the population structure at K= 7, it appears most likely that all the 7 known races of common bean (Singh *et al.,* 1991b) were introduced to Zambia, although this is not altogether conclusive and would require further studies using test genotypes of all the races. Furthermore, there is need to explore the genetic diversity observed based on morphological characters, as seed size and colour are preferred

attributes in the marketing of common bean, and plant growth habits (bush versus climbers) are currently being used to determine the acceptability of new varieties in different locations for different reasons.

Chapter 6

Agro-Morphological Characterisation and Genetic Diversity of Common Bean (*Phaseolus Vulgaris*) Landraces from Zambia

6.1 Introduction

In Zambia common bean (*Phaseolus vulgaris* L.) remains the second most important grain legume after groundnuts in terms area allocated to total legume production and the number of farmers growing it, and is the major source of dietary proteins (Crawford, 1997). In terms of production, Chalwe (2011) showed that beans are produced in all the provinces of Zambia, with the top four provinces accounting for 87.2 percent of bean production (Northern 59.19%, Luapula 10.59%, Central 9.24%, and North Western 8.18%). This increase in bean production since 2004 can be attributed to the government policy of production diversification to include low-input crops like food legumes, which has allowed for a shift from maize dominated production to maize-legumes intercrops hence becoming more appropriate for resource-poor small scale farmers and thereby increasing bean production (Siame *et al*., 1998; Hamazakaza *et al*., 2014). Muimui (2010) noted that common bean is increasingly playing an important role in improving the farmers' livelihoods by providing additional incomes, in addition to food and nutritional security especially to small-scale farmers. The price, scale of operation, distance from the market and the level of mechanisation are among the factors that affect the choice of the marketing channels in Zambia (Chalwe, 2011). Production challenges in Zambia are mainly pests (Bean Stem Maggots), diseases (Angular Leaf Spot, and Bean Anthracnose, Common Bean Bacterial Blight), and unpredictable weather conditions (drought, floods), which have permitted both landraces and improved varieties to circulate in production although landraces dominate in rural communities, and improved varieties in urban agriculture (Katungi *et al*., 2009; Muimui *et al.*, 2011; Hamazakaza *et al*., 2014).

Common beans have been evaluated and screened using different methods ranging from biochemical, molecular, and morphological approaches and their levels of genetic diversity ascertained (Zizumbo-Villarreal *et al*., 2005; Angioi *et al*., 2011; Blair *et al*., 2013a; Blair and Lorigados 2016). The use of morphological traits to evaluate landraces is a traditional, and yet very important method for description and determination of relationships among common bean landraces (Skroch and Niehuis, 1995). Hegay *et al.* (2013) showed that the common bean from Kyrgyzstan clearly were distinguished between the Andean and Mesoamerica using the morphological descriptors, and they further showed that the Andean gene pool were less diverse compared to the Mesoamerican gene pool. Genetic variability in common bean landraces is

greatest as seen in terms of seed and pod colours, patterns, size and shape, with unusual seed and pod colours being an indication of hybridisation among the different landrace sub-populations within the admixture (Martin and Adams, 1987). The seed colour and shape vary from country to country and or region to region and could be a reflection of environmental adaptations and market preferences (Martin and Adams, 1987; Blair *et al*., 2010).

In line with the environmental adaptations, the market classes are defined by seed and pod characteristics and can be broadly classified into dry beans, grown for the mature seed, and snap beans which are grown for the edible pods (Skroch and Niehuis, 1995). In addition, the economic value of a crop population is related to plant morphology, agronomic performance, seed quality and culinary traits (Piergiovanni *et al*., 2000). Therefore, consumers have progressively acquired specific preferences for various combinations of seed traits such as size and shape, and the market reflects this trend by giving preference to good quality types (Negri and Tosti, 2002); hence the need to evaluate materials to cope up with the changes in these market preferences. In addition to these seed and pod characteristics, recently indeterminate beans (climbers) are being preferred over their counterparts the bush beans (Kimani, 2006; Geofrey, 2013). Several reasons have been put forward for this preference ranging from high yields (Blair *et al*., 2010) through nitrogen fixation and nodulation (Graham and Rosas, 1977) to their use in water catchment and soil fertility improvement (Geofrey, 2013).

The goal of this chapter was therefore to evaluate and characterise the Zambian common bean landraces using agro-morphological descriptors. Specifically, this research was undertaken to assess the genetic diversity of landraces of common bean, determine the number of sub-populations for each landrace based on seed characteristics, assign the landraces to the two known gene pools based on these characteristics; and get an insight into the factors responsible for the agro-morphological genetic diversity. Several studies support the use of morphological characters as Awan *et al*. (2014) and Amanullah and Mohammad (2011) showed that there is direct relationship between agro-morphological characters and dry matter production, growth habit and seed per pod, pod length and number of seeds per pod, pod per plant and pod colour intensity, flowering time and ripening time. Participatory plant breeding (PPB) of common beans that involve an integrated approach among farmers, breeders and conservationists rely on these agro-morphological characteristics (Assefa *et al*., 2005; Asfaw *et al*., 2012), and is being promoted as a way of reducing time, number of unacceptable varieties, and increase the number of options available to farmers. Farmers' preferences during PPB such as resistances to abiotic

and biotic stresses measured by plant performance, earliness, marketability, cooking characteristics, seed colour, seed size and growth habits have been documented (Sperling *et al*., 1993; Assefa *et al*., 2005; Asfaw *et al*., 2012). These preferences differ from region to region, and between farmers' categorisation (low income and high incomer farmers) and gender (Sperling *et al*., 1993; Asfaw *et al*., 2012), for instance, women prefer cooking (culinary) characteristics while men prefer marketability. Asfaw *et al*. (2012) noted that breeders should not focus on the traditional colours, size and shape because the bean market is dynamic, and farmers' choices to these characters are not static. Therefore, the use of morphological characters to evaluate and assess genetic diversity of common bean landraces is well justified and is being tested here using the common bean landraces from Zambia.

6.2 Results

6.2.1 Agro-morphological descriptive statistics

The results in this section present descriptive statistics for phenotypic variations in both quantitative and qualitative traits, aimed at answering how useful variation in agro-morphological traits, for characterising common bean landraces in Zambia? The quantitative traits measured were significantly different ($p < 0.05$) among the genotypes studied. For quantitative traits, descriptive statistics such as range, means and their standard errors, phenotypic variance, standard deviation and coefficient of variation were presented in Table 6.1 for all the landrace studies, while a summary of qualitative traits scores are presented in Table 6.2. Agro-morphological traits showed a high level of variation, with quantitative traits such as days to flowering, days to pod maturity, seed volume, seed width, pod length, 100 seed weight, and average seed weight per plant were highest in terms of standard error, standard deviation, phenotypic variance, range and coefficient of variation; hence, these could be more useful in landrace characterisation than others (Table 6.1).

Qualitatively (Table 6.2), majority of the landraces had green (83.06%), purple (14.52%) and red (2.42%) for hypocotyl, stem and leaf venation colour. Plant growth habit was dominated by indeterminate climbers (type IV) (71.77%), although determinate (semi-climbers – type III) (6.45%), determinate bush (type 1) (4.03%) and indeterminate bush (type II) (17.74%) were also present. Four flower types were present in the order of light purple (40.32%), white (37.90%), dark purple (19.35%), and yellow (2.42%). The studied landraces had variations in seed characteristics such as seed venation (present (59.68%), and absent (40.32%)), seed helium ring (present (42.74%), and absent (57.26%)), seed brilliance (mutt (19.35), medium (45.16), and

shiny (35.48%)), seed prominence (darker background (8.87%), lighter background (23.39%), equal darker and lighter background (8.06%), and background absent (59.68%)), and seed shape (round/oval (12.10%), kidney (36.29%), rhomboid/cuboid (9.68%), elongate/cylindrical (20.97%), and truncate/fastigial (20.97%)). There was no dominant seed colour although yellow (26.61%), maroon (16.13%), pinkish stripped (14.52%), brown, pale to dark (8.06%), grey, and brownish to greenish (6.45%), red (5.65%) and purple (5.65%) were recorded. Equally, there were variations the predominant dry pod colour, pod curvature, pod break position and finally position and distribution of pods on the plants (Table 6.2 and Figure 6.1). The combination of quantitative and qualitative variations reported in this section provides very useful information that is needed in the characterisation of these Zambian common beam landraces.

Table 6.1 Quantitative agro-morphological diversity: Range (minimum and maximum), mean, standard error, phenotypic variance, standard deviation, and coefficient of variation values of sixteen quantitative traits for 124 common bean landraces, and the CIAT reference lines studied

Quantitative traits	Range		Mean	Std error	Phenotypic variance	Std. Dev	Coefficient of Variation
	Min	Max					
Days to flowering (DF)	36.00	53.00	46.35	0.26	14.33	3.79	8.17
Days to pod maturity (DPM)	66.00	88.00	79.21	0.40	34.43	5.87	7.41
Seed length - mm (SL)	7.32	18.56	12.88	0.17	5.79	2.41	18.69
Seed width - mm (SW)	5.07	8.93	7.17	0.75	0.56	0.75	10.42
Seed height - mm (SH)	3.25	6.75	5.26	0.05	0.56	0.75	14.22
Seed volume - mm^3(SV)	166.00	978.18	502.32	12.15	31015.24	176.11	35.06
Leaflet length - cm (LL)	14.30	24.40	18.61	0.16	3.83	1.96	10.52
Leaflet width - cm (LW)	7.90	18.40	12.64	0.12	2.87	1.70	13.41
Leaf stalk length - cm (LSL)	8.12	19.40	13.38	0.13	3.59	1.89	14.16
Internode length - cm (IL)	11.30	29.10	18.27	0.31	20.55	4.53	24.81
Average no. of pod $plant^{-1}$ (ANPPP)	4.00	17.00	10.76	0.24	12.53	3.54	32.89
Pod length - mm (PL)	6.90	16.50	11.73	1.04	225.32	15.01	12.80
Pod width - mm (PW)	7.20	26.10	10.85	0.23	10.82	3.29	30.32
Average no. of seeds $plant^{-1}$ (ANSPP)	2.00	9.00	5.33	0.11	2.09	1.45	27.09
100 Seed weight - g (100SW)	17.11	48.25	32.83	0.50	52.57	7.25	22.08
Average seed weight $plant^{-1}$ (ASWPP)	5.09	25.45	14.66	0.42	37.68	6.14	41.88

Table 6.2 Qualitative Variations in the 124 common bean landraces based on the scored fourteen qualitative traits used in this study

Qualitative trait	Scored parameter	Percentage
Hypocotyl colour	Green	83.06
	Pink	14.52
	Red	2.42
Stem colour	Green	83.06
	Pink	14.52
	Red	2.42
Colour of Leaf Venation	Green	83.06
	Pink	14.52
	Red	2.42
Plant Growth Habit	Determinate Bush	4.03
	Indeterminate Bush	17.74
	Determinate Prostrate	6.45
	Indeterminate Prostrate	71.77
Flower colour	White	37.90
	Light Pink	40.32
	Dark Pink	19.35
	Yellow	2.42
Dry Pod Colour	Dark Purple	3.23
	Carmine red	4.03
	Purple stripe on yellow	16.94
	Dark Pink	9.68
	Pale yellow to white	36.29
	Golden or deep yellow	29.84
Pod Break Position	Central	41.13
	Placental	58.87
Pod Curvature	Straight	41.13
	Slightly curved	36.29
	Curved	18.55
	Recurving	4.03
Position of the pods on the plant	Base	0.00
	Centre	0.00
	Top	12.10
	Combination of all	87.90
Seed Shape	Oval/Round	12.10
	Kidney	36.29
	Rhomboid/cuboid	9.68
	Elongate/cylindrical	20.97
	Truncate/fastigial	20.97
Seed Colour	White, purple tinged	3.23

	Brown, pale to dark	8.06
	Pale cream to buff	0.81
	Black	1.61
	Purple	5.65
	Yellow	26.61
	Red	5.65
	Maroon	16.13
	Green to Olive	4.84
	Chlorophyll green	0.81
	Whitish	1.61
	Pure white	4.03
	Grey, brownish to greenish	6.45
	Pinkish-stripped	14.52
Seed Brilliance	Matt	19.35
	Medium	45.16
	Shinny	35.48
Seed Venation	Absent	59.68
	Present	40.32
Seed Prominent	Darker Backgrounds with lighter stripes	8.87
	Lighter background with darker stripes	23.39
	Dark background is equal to light background	8.06
	Absent	59.68
Helium ring	Present	42.74
	Absent	57.26

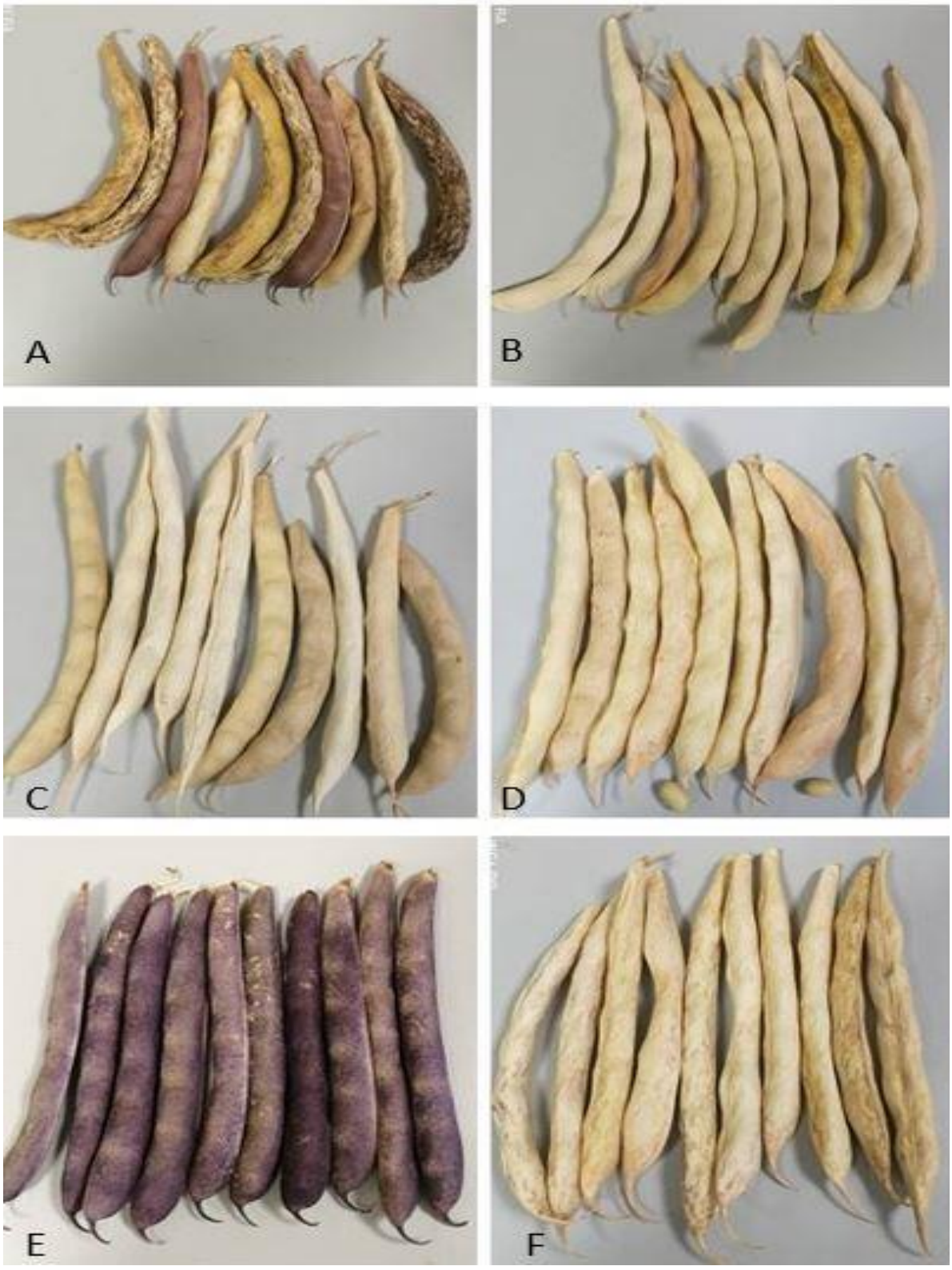


Figure 6.1 Pod colour and shape diversity among common bean landraces from Zambia: A – Solwezi, B – Mbala Mixture, C – Lundazi, D – Lusaka Yellow, E and F – CIAT reference line G5773, and G14770 respectively. Pod admixture and diversity evident for landraces A-D compared to pure CIAT reference lines E and F.

6.2.2 Pairwise correlations of quantitative traits

Pairwise correlations of the 16 quantitative traits produced strong and positive correlations as well as weak and negative correlations (Table 6.3). In plant breeding, knowledge of pairwise correlations of quantitative traits is important to breeders as quantitative traits tend to be polygenic in nature. In this current study, the results showed that there are strong positive correlations ($p < 0.05$) amongst different pairs such as seed length and/or seed width to days-to-pod-maturity; seed height, leaf length, leaf stalk length, internode length, pod length, 100 seed weight, and average seed weight per plant to days to flowering; seed volume to seed height; and seed width and/or seed height to leaf length. Further strong positive correlations existed amongst seed length, seed width, seed height, leaf width, and leaf stalk length to Internode length; seed volume, leaf length, leaf width, and leaf stalk length to average number of pod per plant; days to pod maturity, seed length, seed height, seed volume, and leaf length to pod length; days to pod maturity, seed length, seed width, seed height, and pod length to average number of seed per plant; and seed length, seed width, leaf length, pod length, pod width, and 100 seed weight to average seed weight per plant (Table 6.3). A strong negative correlation was observed between days to flowering and average number of pods per plants. For traits that are positively correlated to one another, it implies that improving one trait will automatically improve the other trait, and for traits that are negatively correlated, it implies that improving one traits will negatively affect the other trait.

Table 6.3 Pairwise correlations between the quantitative traits: days to flowering (DF), days to pod maturity (DPM), seed length (SL), seed width (SW), seed height (SH), seed volume (SV), leaflet length (LL), leaflet width (LW), Leaflet stalk length (LSL), internode length (IL), average number of pod per plant (ANPPP), pod length (PL), pod width (PW), average number seed per pod (ANSPP), 100 seed weight (100SW), and average seed weight per plant (ASWPP) measured on the 120 individuals of the four landraces and 4 individuals of one each for the CIAT reference lines

	DF	**DPM**	**SL)**	**SW**	**SH**	**SV**	**LL**	**LW**	**LSL**	**IL**	**ANPPP**	**PL**	**PW**	**ANSPP**	**100SW**	**ASWPP**
DF		0.372	0.989*	0.542*	0.792*	0.279	0.044	0.723*	0.805*	0.691*	0.612*	0.489	0.395	0.229	0.314	0.774
DPM	0.372		0.337	0.147	0.292	0.047	0.004	0.697*	0.294	0.697*	0.778*	0.092	0.098	0.041	0.055	0.615*
SL	0.989*	0.337		0.666*	0.935*	0.279	0.037	0.552*	0.972*	0.521*	0.453	0.446	0.458	0.270	0.312	0.614*
SW	0.542*	0.147	0.666*		0.754*	0.559*	0.121	0.344	0.815*	0.384	0.266	0.830*	0.672*	0.498	0.550*	0.384
SH	0.792*	0.292	0.935*	0.754*		0.398	0.077	0.579*	0.900*	0.578*	0.465	0.630*	0.525*	0.337	0.420	0.551*
SV	0.279	0.047	0.279	0.559*	0.398		0.335	0.126	0.404	0.146	0.087	0.647*	0.863*	0.909*	0.973*	0.158
LL	0.044	0.004	0.037	0.121	0.077	0.335		0.016	0.083	0.018	0.010	0.144	0.304	0.379	0.333	0.021
LW	0.723*	0.697*	0.552*	0.344	0.579*	0.126	0.016		0.520*	0.945*	0.902*	0.225	0.244	0.119	0.152	0.975*
LSL	0.805*	0.294	0.972*	0.815*	0.900*	0.404	0.083	0.520*		0.474	0.424	0.607*	0.630*	0.366	0.4334	0.4896
IL	0.691*	0.697*	0.521*	0.384	0.578*	0.146	0.018	0.945*	0.474		0.927*	0.233	0.236	0.113	0.142	0.990*
ANPPP	0.612*	0.778*	0.453	0.266	0.465	0.087	0.010	0.902*	0.424	0.927*		0.164	0.195	0.085*	0.110	0.880*
PL	0.489	0.092	0.446	0.830*	0.630*	0.647*	0.144	0.225	0.607*	0.233	0.164		0.790*	0.658*	0.685*	0.276
PW	0.395	0.098	0.458	0.672*	0.525*	0.863*	0.304	0.244	0.630*	0.236	0.195*	0.790*		0.7861*	0.904*	0.231
ANSPP	0.229	0.041	0.270	0.498	0.337	0.909*	0.379	0.119	0.366	0.113	0.085	0.658*	0.786*		0.912*	0.120
100SW	0.314	0.055	0.312	0.550*	0.420	0.973*	0.333	0.152	0.433	0.142	0.110	0.685*	0.904*	0.912*		0.161
ASWPP	0.774*	0.615*	0.614*	0.384	0.551*	0.158	0.021	0.975*	0.490	0.990*	0.880*	0.276	0.231	0.120	0.161	

*positive and significant linear correlations ($p<0.05$)

6.2.3 Use of qualitative traits to access genetic diversity of common bean landraces

The genetic diversity of the common bean landraces was accessed using the 14 qualitative traits (Table 6.4). The diversity parameters accessed included number of alleles (Na), effective number of alleles (Ne), Nei's gene diversity (h), Shannon information Index (I), and percentage of polymorphic loci (% loci) and the results show that there was a high genetic diversity among the four landraces of Lusaka yellow, Lundazi, Mbala, and Solwezi beans with very small variation within them based on qualitative traits. Genetic diversity was highest in Lundazi, Mbala mixture, Solwezi and Lusaka yellow in that order, and the percentage of polymorphic loci followed that same trend too.

Table 6.4 Mean of genetic diversity parameters: Number of individual (N), Number of alleles (Na), Effective number of alleles (Ne), Nei's gene diversity (h), Shannon information Index (I), and percentage of polymorphic loci (% loci) based on 124 individuals and 14 qualitative traits

Landraces	**N**	**Na**	**Ne**	**h**	**I**	**% loci**
Lusaka Yellow	30	1.4844	1.2383	0.279	0.425	48.44
Lundazi	30	1.6250	1.3514	0.331	0.499	62.50
Mbala Mixture	30	1.6875	1.3625	0.325	0.498	68.75
Solwezi	30	1.6875	1.3230	0.292	0.453	68.75
CIAT Reference	04	0.9658	0.9618	0.099	0.146	46.88
Mean	**24.8**	**1.49004**	**1.2474**	**0.2652**	**0.4042**	**59.064**

6.2.4 Highly structured populations of the Zambian common bean landraces based on quantitative and qualitative traits

Agro-morphological traits were used to assess the population structure of the Zambian common bean landraces. Qualitative traits based on seed types (colour, size and shapes) identified several sub-populations under each landrace are presented in Figure 6.2A-D and Annex 3. The overlaps amongst the different landraces and the commercial varieties mixed within each landrace could also be identified. Overall, based on these seed types, Lusaka yellow had 11 subpopulations, Lundazi 12, Mbala mixture 25 and Solwezi 23. It is also important to report that a higher number of commercial varieties could be seen in the Mbala mixture and Solwezi landraces. Principal component analysis (PCA) based on qualitative, quantitative and a combination of these traits was sufficient to identify the most descriptors to characterise these landraces, and clearly separated the landraces into Andean and Mesoamerican gene pools.

The first three component of the quantitative traits accounted for 28.87% of the variance, 12.61% was accounted for by the qualitative traits, and 11.08% by the combination of these traits (Table 6.5). For quantitative traits, principal component 1 (PC1) identified seed width, pod length, leaf stalk length, internode length, and seed volumes as most descriptive traits; PC2 identified seed height, seed weight per plant, number of seed per plant, seed length, and average number of pod per plant; and PC3 identified average number of pod per plant, seed weight per plant, number of seed per plant, 100 seed weight and pod length. Therefore, of the 16 quantitative traits, 11 traits can be regarded as most descriptive, while the other 5 as redundant traits, and that introducing more traits in the agro-morphological studies introduces redundancy and affects the overall percentages that are explained by these traits.

Table 6.5 Variation in Agro-morphological (quantitative, qualitative and combination) traits in accounting for the observed variation in the Zambian common bean landraces. Quantitative traits accounted highly for the total variance followed by qualitative, and lowest by combined traits.

Principal Component	Quantitative traits			Qualitative traits			Combined traits		
	Eigenvalue	% variance	% Cummulative	Eigenvalue	% variance	% Cummulative	Eigenvalue	% variance	% Cummulative
1	1.73774	10.753	10.753	2.83086	4.4556	4.4556	3.05898	3.8383	3.8383
2	1.52348	9.4271	20.1801	2.68013	4.2184	8.674	2.97812	3.7369	7.5752
3	1.40422	8.6891	28.8692	2.49977	3.9345	12.6085	2.79317	3.5048	11.08
4	1.32406	8.1931	37.0623	2.36562	3.7233	16.3318	2.60817	3.2727	14.3527
5	1.2607	7.8011	44.8634	2.29584	3.6135	19.9453	2.52641	3.1701	17.5228
6	1.168	7.2274	52.0908	2.15815	3.3968	23.3421	2.4127	3.0274	20.5502
7	1.11914	6.925	59.0158	2.13042	3.3531	26.6952	2.32947	2.923	23.4732
8	1.03095	6.3794	65.3952	2.00066	3.1489	29.8441	2.28983	2.8732	26.3464
9	0.969834	6.0012	71.3964	1.93396	3.0439	32.888	2.25549	2.8301	29.1765
10	0.856186	5.298	76.6944	1.78861	2.8152	35.7032	2.20378	2.7652	31.9417
11	0.763566	4.7248	81.4192	1.77377	2.7918	38.495	2.08397	2.6149	34.5566
12	0.758397	4.6928	86.112	1.69551	2.6686	41.1636	2.00507	2.5159	37.0725
13	0.671639	4.156	90.268	1.60964	2.5335	43.6971	1.92868	2.4201	39.4926
14	0.602456	3.7279	93.9959	1.54806	2.4365	46.1336	1.86898	2.3451	41.8377
15	0.538759	3.3338	97.3297	1.53398	2.4144	48.548	1.81353	2.2756	44.1133
16	**0.431553**	**2.6704**	**100.0001**	**1.51217**	**2.3801**	**50.9281**	**1.73999**	**2.1833**	**46.2966**

Figure 6.2A Subpopulations of Lusaka yellow beans landrace revealed by seed types. Eleven sub-populations were evident with some overlaps in Mbala mixture landrace.

Figure 6.2B Sub-populations of Lundazi beans landraces revealed by seed types. Twelve sub-populations were evident with some overlaps in Mbala mixture and Solwezi landraces.

Figure 6.2C Sub-populations of Mbala mixture beans landrace revealed by seed types. Twenty five sub-populations were evident with some overlaps in Lusaka yellow, Lundazi, and Solwezi landraces.

Figure 6.2D Sub-populations of Solwezi beans Landrace revealed by seed types. Twenty five sub-populations were evident with some overlaps in Lusaka yellow, Lundazi, and Solwezi landraces.

Clustering analysis of agro-morphological traits based on Euclidian distances and using the Neighbour-joining method clearly separated the Andean and Mesoamerican genepools (Figure 6.3) using PAST software. Based on this result, the Mesoamerican genepool dominates the Andean, and a high proportion of the Mesoamerican genepool are products of hybridization (commercial varieties). The Mesoamerican further separated into two main clusters of landraces and commercial varieties, and within these two main sub-clustering, there were further clustering, which is a reflection of the sub-population within these landraces. Analysis of sub-populations based on qualitative traits using STRUCTURE software and implementing Elvanno's method, identified K12 as the appropriate number of sub-populations in these Zambian landraces (Figure 6.4) and further analysis of population structure produced results similar to clustering pattern (Figure 6.5). Specifically, figure 6.5 shows that the Zambian landraces is dominated by the Mesoamerican genepool (K2), and that this genepool has a high proportion of commercial materials in it (K3).

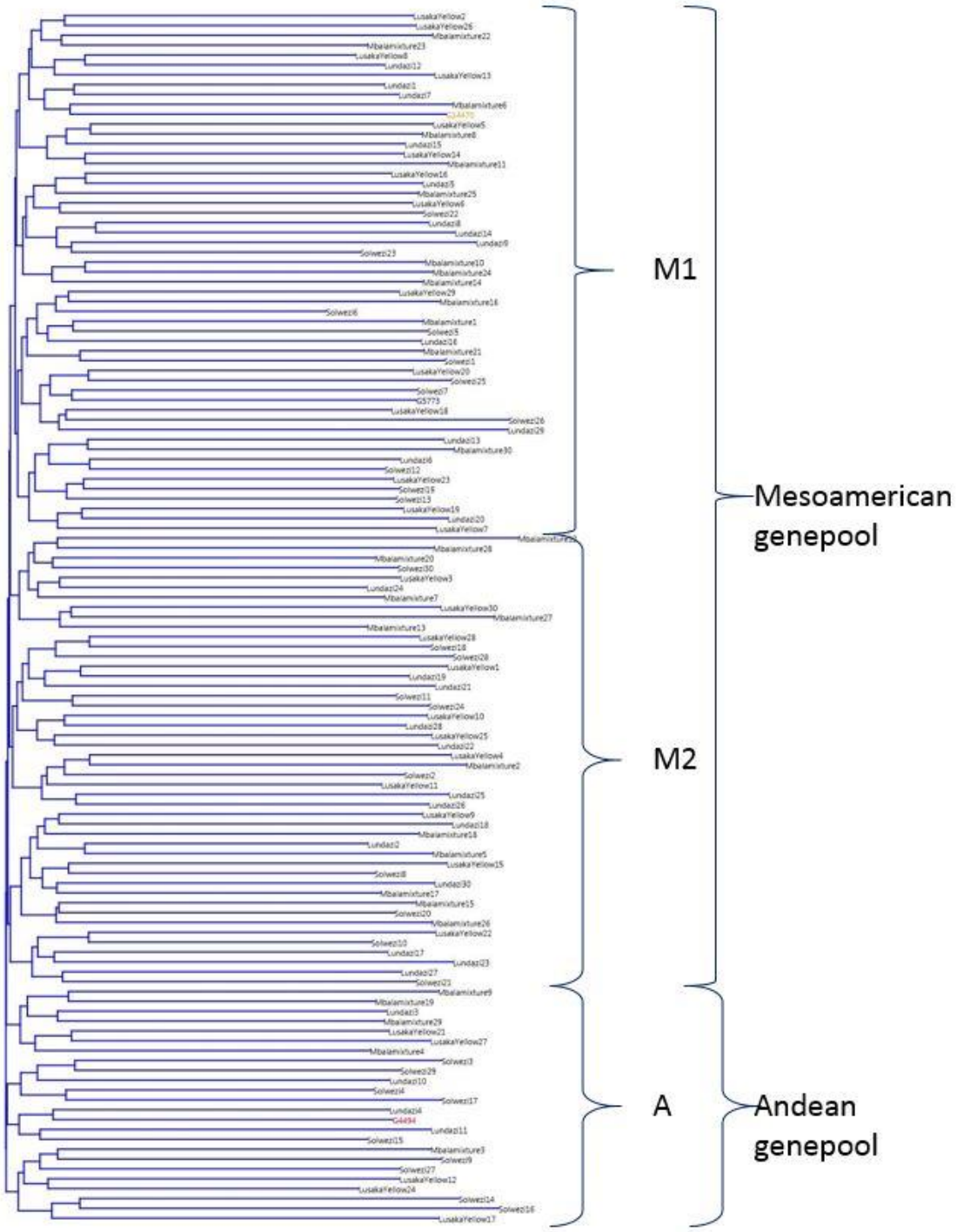


Figure 6.3 Agro-morphological clustering based on Euclidian distances and using Neighbor joining method clearly separated the Andean (A) amd Mesoamerican (M) genepools. Mesoamerican gene pool predominates based on Agro-morphological traits and is sub-divided into products of hybridization (M1) and landraces (M2).

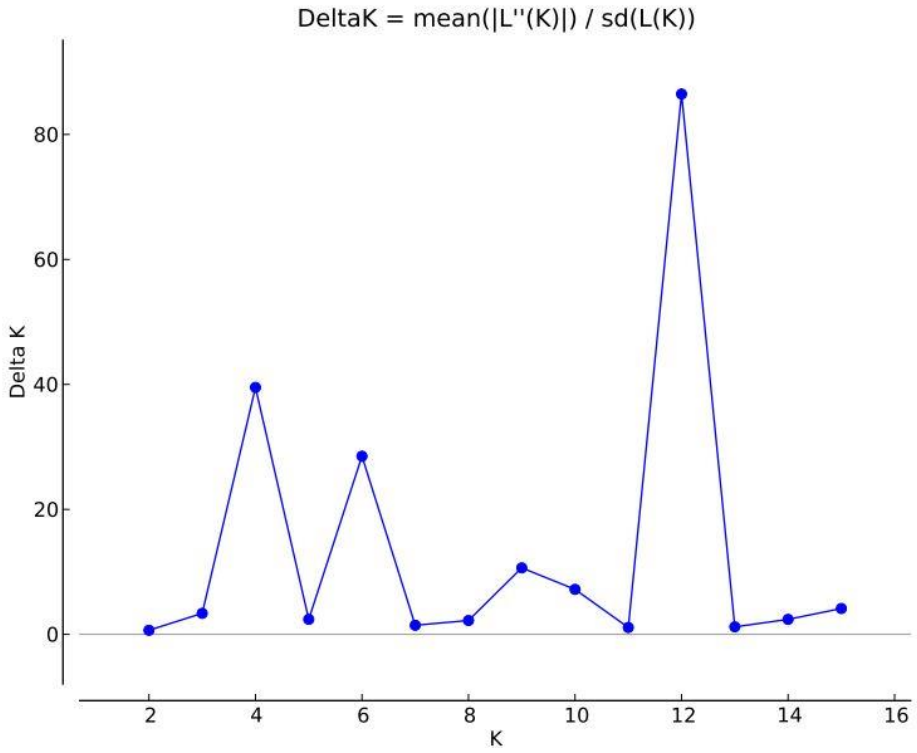


Figure 6.4 Agromorphological estimation of the optimum number of sub-population for the Zambian Landraces according to the Elvanno's method implemented by Structure Harvester as described by Earl and vonHoldt (2012). The graph displays the DeltaK [mean(|L"(K)|)/SD(L(K))] for each K value, and the highest K values is the optimum number of sub-populations, which in this study is 12, although 4 and 6 are also high.

An estimation of the optimal number of sub-populations in the Zambian common bean landraces according to Elvanno's method produced peaks at K4, K6, K9, K12, and K15, although the highest peak was at K12. This demonstrate very highly structured populations within these landraces, consisting of both Andean and Mesoamerican genepools, and the commercial varieties that could have resulted from the hybridisation of these genepools.

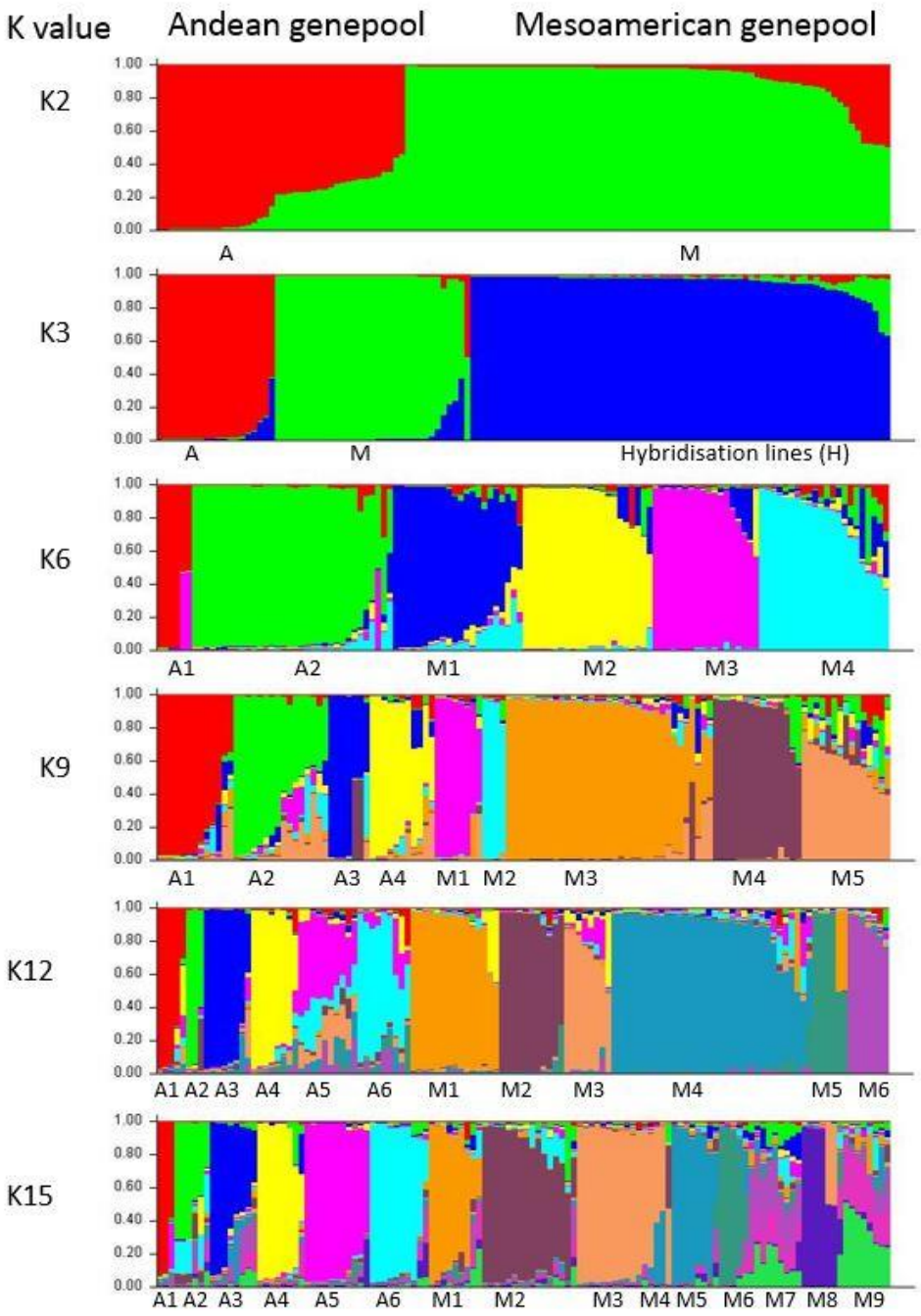


Figure 6.5 Agro-morphological population structure at K2, K3, K6, K9, K12, and K15 for 124 individuals of the Zambian common bean landraces and the CIAT referenced lines. The different K values are the assumed populations. The distinction between Andean and Mesoamerican genepools (K2); Introgression between the two genepools at K3 (Hybridisation lines - H); Andean and Mesoamerican subgroups at K6 (Andean 2 sub-populations and Mesoamerica 4 sub-populations); K9 (Andean 4 sub-populations and Mesoamerica 5 sub-populations); K12 (Andean 6 sub-populations and Mesoamerican 6 sub-populations); and K15 (Andean 6 sub-populations and Mesoamerica 9 sub-populations). The vertical lines represent the individuals in the STRUCTURE bar diagrams, while the different colours of each bar line represent the genetic composition of that individual.

6.3 Discussion

The main objective of this chapter was to investigate the extent to which agro-morphological traits can be used to characterise common bean landraces and assess their genetic diversity. Use of quantitative, qualitative or a combination of these traits provided very useful information in relation to common bean landrace characterisation, as well as assessing their genetic diversity. Qualitatively based on seed types, the sub-populations of different landraces were identified with Mbala mixture having the highest number (25), Solwezi (23), Lundazi (12), and Lusaka yellow (11). Using the Zambian bean variety descriptors (Muimui, 2015), the commercial varieties mixed in these landraces were identified with varying numbers. Lusaka yellow has one commercial variety (Lwangeni), Lundazi (03) – Mbereshi, Kabale and Kabulangeti, Mbala mixture (05) – Chambeshi, Lukupa, Kalungu, Kabulangeti, and Lunga, and Solwezi (05) – Lukupa, Kalambo, Kaprisha, Lungwebungu, and Lunga. The high number of sub-populations observed in this study could be attributed to the presence of commercial varieties that could have come from different genetic backgrounds that has resulted from seed admixture and their modest outcrossing rates, as explained in chapter 5 of this thesis. The combined qualitative traits showed that there is a high genetic diversity amongst these landraces with the highest being Lundazi (0.499), Mbala Mixture (0.498), Solwezi (0.453), Lusaka yellow (0.425), and the CIAT reference lines (0.146) in that order, with an average Shannon diversity index of 0.404. The order of diversity levels within the landraces vary between the molecular and agro-morphological methods, but show clearly that Lusaka yellow is the least diversified landrace. The variation between these methods could be attributed to sample size difference, and random sampling procedure during individual selections under each landrace.

These results are comparable with others from the use of agro-morphological traits to assess diversity in common bean landraces, for example, Hegay *et al*. (2014) found a low diversity index of 0.05 when they studied the genetic diversity of the Kyrgyzstan common bean varieties using qualitative traits, while Okii *et al*. (2014a) found a high diversity index of 0.56 when they studied the genetic diversity on the tropical common bean germplasm from Uganda; and Hamazakaza *et al*. (2014) and Burle *et al*. (2011) showed that the Zambian and Brazilian farmers respectively, deliberately mixed both landraces and commercial varieties in the same piece of land during production. These results show that agro-morphological traits are appropriate to assess the genetic diversity of common bean landraces, and the differences in the results could be attributed to the source of the germplasm, genepool composition of the samples, and the choice of agro-morphological traits, that is, qualitative, quantitative or both. Chiorato *et al*. (2006) used

a combination of agro-morphological and molecular markers to identify duplicates in the Brazilian common beans and found 100-seed-weight less informative as they ranged from 20 – 25g/100seeds. Their result could be supported by the fact that Brazil is dominated by Mesoamerican beans and hence there would be no difference in 100 seed weight. For this same reason, the 100 seed weight was very useful in characterising the common bean landraces from Zambia since both the genepools are present in these materials with a wide range in 100 seed weight of 17.11 – 48.25g on average.

Pairwise correlations between the quantitative traits produced both positive and negative correlations (Table 6.3). These correlations between quantitative traits provide useful information to the common bean breeders when it comes to genetic improvement of these traits. Similar correlations (positive and negative) for common bean were reported by Okii *et al*. (2014a) in their study tropical bean germplasm in Uganda, and Awan *et al*. (2014) in their study of genotypes under rain fed conditions in Pakistan. As Okii *et el.* (2014a) explained, strong positive correlation between traits allows for simultaneous selection, their use in common bean improvement interchangeably, and that these traits are probably under the influence of same gene or pleiotropic effects. For instance, in this study we observed strong positive correlations between pod length and days to flowering (r = 0.933), days to flowering to average seed weight per plant (r = 0.902), and leaflet width and average seed weight per plant (r = 0.950). These results mean that, for example, selecting days to flowering as a breeding trait would simultaneously lead to selection of pod length and average seed weight per plant, while selecting leaflet length would also lead to selecting average seed weight per plant. In the latter case, leaflet length has a direct influence on the amount of solar radiation that is captured as well as the amount of photosynthetic product that is partitioned to the developing seeds during the grain filling phase of plant development. The relationship between pod length and number of seeds per plants as well as seed yields was reported earlier (Cakmakci *et al*., 2003; Assefa *et al*., 2005; Asfaw *et al*., 2012), and that farmers tend to rely on these traits to select high yielding common bean varieties.

A negative correlation was observed between among certain pair of traits during this analysis. This result means that selecting plants with early flowering would result in fewer numbers of pods per plant. The probable explanation for this observation is that early flowering common bean plants do so when they haven't put on enough vegetative structures to support the high number of flowers, hence abortion would result and thus few pods. This strong negative correlation is not peculiar to common beans as Abaca *et al*. (2012b) observed the same in cassava

between root yields and root dry matter. Therefore, the selection of such traits with strong negative correlations should be conducted judiciously, as attempting to advance both will affect the acceptability of the products to the end users. A near unit correlations was also observed between 100 seed weight and days to pod maturity (r = 0.962), 100 seed weight and average weight of seed per plant (r = 0.961), and leaflet width and seed height (r = 0.957). Okii *et al*. (2014a) attributed this near to unit correlations between traits to their being controlled by one gene or they are closely linked.

The principal component analysis (PCoA) based on Agro-morphological traits partitioned the studied common bean landraces into two main clusters representing the Andean and Mesoamerican genepools, with the majority of individuals clustering at the midpoint of the two axes. Besides placing the studied landraces into their respective genepools, PCA was also used to identify the most informative agro-morphological traits that can be used in common bean characterisations. In this current study, principal component one (PC1) identified days to flowering, seed height, seed volume, pod length, number of pods per plant, 100 seed weight, weight of seed per plants, seed venation, plant growth habit, flower colour, pod dry colour, seed colour, seed brilliance, and seed prevalent as key components. PC2 identified days to flowering, seed with, days to pod maturity, Leaf stalk length, number of seeds per pod, weight of seed per plants, hypocotyl colour, plant growth habit, flower colour, dry pod colour, pod curvature, seed shape and seed colour as its key components and finally PC3 identified days to flowering, seed length, seed volume, leaflet length and width, number of pods per plant, 100 seed weight, and weight of seed per plant as the most informative traits that can be used to characterise common bean. This result agrees with those of Okii *et al*. (2014a), Asfaw *et al*. (2009; 2012), and Assefa *et al*. (2005) on their studies on common bean from East African highlands regions that identified days to flowering, days to pod maturity, pod length, number of seeds per pod, plant growth habit, and flower colour as appropriate in characterising common bean, and that farmers rely on them frequently during participatory plant breeding evaluations. This further supports the observation by Singh *et al*. (1991a) that variation in agro-morphological characters in common bean could be independent variables, and that same agro-morphological pattern can be found in different genepools. This result means that although all the agro-morphological traits measured can be used in germplasm characterisation and diversity analysis, adequate information can also be generated by focusing on only few of these agro-morphological traits identified by the PCA above to variation among the landraces and characterised them. Chiorato *et al*. (2005) had previously shown that only 18 of the 25 agro-morphological traits were needed to evaluate common bean accessions. This supports the results presented in Table 6.5, that either

quantitative, qualitative or both agro-morphological traits should be used carefully as more traits introduces redundancy in the evaluation and hence different results from the same data set.

A population structure analysis using qualitative traits grouped the landraces into the Andean and Mesoamerican genepools at K2 that supports results of the PCA, and at K3 hybridizations between the genepools are represented. Analysis of sub-populations within these materials using the Evanno's method implemented in the STRUCTURE HARVESTER by Earl and vonHoldt, (2012) produced peaks at different K values of 4, 6, 9, 12 and 15, with K12 being the highest peak and thus 12 sub-populations present within these landraces. This result confirms the high population structure within the Zambian landraces that was reported using the single sequence repeat (SSR) markers approach in Chapter 5. This was far higher than those reported previously in the East African region (Okii *et al*., 2014a; Asfaw *et al*., 2009), and was closely comparable to those reported in Brazil (Burle *et al*., 2010 and 2011). This high population structure is a reflection of high genetic diversity within the Zambian landraces, which is attributed to the seed admixture, original introductions, varying biophysical factors (soils, climate, altitude), and porous border points with Angola, Democratic Republic of Congo (DRC), Tanzania, and Malawi (top 10 bean producers in Africa) thus making placing Zambia as a secondary centre for bean diversity in Southern Africa.

This research has demonstrated throughout farmers' farming practices alongside environmental conditions are key in shaping up the seed types admixture and their overlaps. This research has also demonstrated the importance of phenotypic variations especially seed types to identify the marketing channels. Combining Chapters 4 and 6 it is clear that seed size doesn't matter in Zambia but seed colour which is a reflection uniformity does. These agro-morphological traits have confirmed that the common bean landraces from Zambia have a higher proportion of the Mesoamerican genepool, and that most commercial varieties in Zambia is mainly from this genepool too that strongly supports results from Chapter 5. If the observations by Broughton *et al*. (2003) that 60 percent of bean occurs in drought prone environment, and by White and Singh (1991) and Beebe *et al*. (2013) that sources of drought tolerance were discovered in small seeded beans of Durango and Mesoamerican races, then this result confirms the dominance of Mesoamerican genepool in Zambia to be associated with their tolerance to drought conditions.

Chapter 7

Assessment of Molecular and Agro-morphological Changes in the Common Bean Landraces over the three Growing Seasons (2014-2016)

7.1 Introduction

The genetic diversity of the landraces of common beans from Zambia have been dealt with thoroughly in Chapters 5 and 6 using molecular and agro-morphological approaches. Among the different mechanisms that has been put forward in explaining the genetic diversity and population structure observed in these chapters as mutation, selection, and migration of both seed and pollen (Papa, 2005). Selection results from the interaction of the demand and supply in the market, together with effects of agroecosystems and the decisions of farmers, these all have tremendous impact on the resulting diversity and population structure of the crop in question. Common bean being an annual crop that is generally self-pollinating (Weinstein, 1926); however, although different levels of outcrossing have been reported (Gepts and Papa, 2003; Papa and Gepts, 2003; Papa, 2005; Beebe *et al*., 1997; Ferreira *et al*., 2007), ranging from 0.2 to 1.4 percent. Higher rates of outcrossing have been reported in some particular environments; for example, Pompeu (1963, cited in Ferreira *et al*., 2007) and Brunner and Beaver (1988) showed a rate of 6.0% and 17.6% for common beans in Brazil and USA respectively. The presence of wild-weedy-crop complexes was also reported in Peru and Colombia to signify the outcrossing between domesticated and wild common bean (Beebe *et al*., 1997) under natural conditions. Beebe *et al*. (1997) and Ferreira *et al*. (2007) pointed out that, there are several reasons to explain this huge variation in the rate of outcrossing in common bean including: i) different methods used to create natural hybridization and the evaluation of the inbred lines, ii) planting season that involves wet and dry seasons, iii) presence of pollinating insect species, and iv) the farming system. Specifically, Vieira (1960) and Pacova and Rocha (1975), all cited in Ferreira *et al*. (2007), showed that there is a higher rate of outcrossing in beans during dry and wet seasons by 0.18% in dry seasons for both studies, and 0.70% and 0.35% respectively for wet season.

Symmetric and Asymmetric models have been used to understand the mechanisms of gene flow in common beans (Papa and Gepts, 2003; Papa, 2005). The Symmetric model assumes equal population size and rate of gene flow between the sub-populations that affects the structure of genetic diversity by homogenisation of the allele frequencies among sub-populations but will not affect total genetic diversity. The Asymmetric model assumes different population sizes and rates of gene flow between the sub-populations, with the ultimate effect of reducing the genetic

diversity of the recipient population but with no effect on population structure (Papa, 2005). Papa and Gepts (2004) studied the gene flow between domesticated landraces and the wild populations of common bean. They noted, a higher gene flow from domesticated to wild populations than the reverse. However, they pointed the two factors of population size, and the selection pressure from both the agroecosystems and farmers, as being key in determining the direction of gene flow.

Outcrossing rates, which are measured in terms of gene flow, are known to have positive (increases) or negative (decreases) effects in the genetic diversity of the crop in question (Ellstrand *et al*., 1999). Therefore, the study of outcrossing rates in common bean is very important in the area of plant breeding and genetic conservation as it is important in achieving seed purity standards and setting up of isolation distances required for these purposes. The components of distinctness, uniformity and stability are important parameters in registration of a new variety (Jones *et al*., 2003; CPVO, 2013), including landrace registration. In the case of the Zambian landraces, this component of outcrossing rate is important in evaluating the rate at which these landraces may change over time (both genetic diversity and population structure) following their registration, and subsequent production and utilization.

This chapter therefore presents the molecular and agro-morphological changes in the Zambian common bean landraces over three growing seasons of 2014 designated as A, 2015 as B and 2016 as C in the result sections. This study focuses on the allele frequencies, estimated gene flow, the actual allele sizes by SSR marker and populations, and analysis of molecular variance (AMOVA) to establish molecular changes that could have occurred in these landraces over these the growing seasons (Beals *et al*., 2000a,b). At agro-morphological levels changes in flower colour, seed types (colour, size and shapes), seed length and width, and number of sup-populations within each of the three growing seasons were used to assess the levels of these changes.

7.2 Results

7.2.1 Recap of Genetic diversity over the three growing seasons

The measure of genetic diversity as indicated by Shannon index, expected heterozygosity, and unbiased expected heterozygosity, showed that it was high, although it decreased from 2014 to 2016, and that landraces had higher diversity indices than the CIAT reference lines over the three growing seasons (Table 7.1). The genetic diversity as a whole for the landraces was highest in Mbala mixture (0.486), followed by Solwezi, Lundazi, and lowest in Lusaka yellow (0.297). For the CIAT reference line, it was highest in G14470 (0.248, a Zambian commercial line), through G9794, G22493, G19833, G5773 and lowest in G4494 (0.177). The highest value for genetic diversity among the CIAT reference lines was still lower than the lowest value for the Zambian landraces. On average, the genetic diversity decreased not significantly from 2014 (0.436) to 2016 (0.408) for the landraces, although this was not the case for the CIAT lines. There was no clear trend for the number private alleles as its values stood at 0.489, 0.429 and 0.739 for 2014, 2015 and 2016 growing seasons respectively for the landraces, and these values were higher than for their CIAT reference lines counterparts.

Table 7.1 Summary of allelic parameters used to measure genetic diversityover the three growing seasons: Shannon index (I), number of private alleles (PA), expected heterozygosity (He), and unbiased expected heterozygosity (uHe) by populations (Landraces and CIAT lines) and growing seasons (2014-2016)

Year	Allelic Parameters	Populations										Mean for Landraces	Mean for CIAT Lines
		Landraces				CIAT Reference Lines							
		LusakaY	Lundazi	Mbala M	Solwezi	G9794	G5773	G4494	G19833	G14770	G22493		
2014	I	0.502	0.829	1.072	0.976	0.580	0.556	0.538	0.619	0.825	0.440	0.845	0.593
	PA	0.727	0.591	0.318	0.318	0.000	0.045	0.000	0.091	0.091	0.000	0.489	0.038
	He	0.258	0.415	**0.545***	0.510	0.351	0.325	0.333	0.375	**0.499***	0.259	0.432	0.357
	uHe	0.260	0.418	0.550	0.515	0.381	0.354	0.364	0.406	0.549	0.283	0.436	0.390
2015	I	0.475	0.882	0.880	0.895	0.208	0.159	0.126	0.159	0.153	0.241	0.783	0.174
	PA	0.429	0.476	0.429	0.381	0.000	0.000	0.000	0.095	0.000	0.000	0.429	0.016
	He	0.237	**0.479***	0.468	0.465	0.143	0.113	0.089	0.113	0.107	**0.167***	0.412	0.122
	uHe	0.239	0.485	0.475	0.471	0.206	0.151	0.151	0.167	0.159	0.222	0.418	0.176
2016	I	0.723	0.756	0.842	0.680	0.262	0.183	0.152	0.183	0.209	0.293	0.750	0.214
	PA	0.955	0.818	0.682	0.500	0.045	0.045	0.000	0.045	0.045	0.045	0.739	0.038
	He	0.394	0.390	**0.446***	0.380	0.182	0.131	0.108	0.131	0.136	**0.193***	0.403	0.147
	uHe	0.403	0.394	0.451	0.386	0.273	0.174	0.174	0.189	0.197	0.258	0.408	0.211
Mean	I	0.567	0.823	0.931	0.851	0.350	0.299	0.272	0.320	0.395	0.325	0.793	0.327
	PA	0.703	0.628	0.476	0.400	0.015	0.030	0.000	0.077	0.045	0.015	0.552	0.031
	He	0.297	0.428	**0.486**	0.452	0.225	0.190	0.177	0.206	**0.248**	0.206	0.416	0.209
	uHe	0.301	0.432	0.492	0.457	0.287	0.226	0.230	0.254	0.302	0.254	0.421	0.259

*the highest value for genetic diversity by population and by growing season

7.2.2 Molecular changes

In an attempt to quantify the amount and significance of molecular changes across the landrace populations and growing seasons, the allele frequencies and their changes over the growing seasons (Table 7.2), the estimated gene flow (Nm) and their changes (Table 7.3), the actual allele size by locus and growing season (Annex 3), changes in inbreeding coefficient (Table 7.4), changes in population structure (Figure 7.1) and the analysis of molecular variance over the growing seasons (Figure 7.2) are presented.

Analysis of variance for allele frequencies, number of alleles, and estimated gene flow showed that these values were not significantly different ($p < 0.05$) over the three growing seasons. The same applies for their changes over the three growing seasons. Allelic frequency decreased from 2014 (0.205) to 2016 (0.174), with more variation being recorded in 2014 and 2015 compared to 2016. The estimated gene flow (Nm) showed a similar pattern to allele frequency, that is, it decreased from 2014 (0.458) to 2016 (0.181) with more variation in 2016 than 2015 and 2014.

Table 7.2 Allele frequencies and their changes over the three growing seasons of 2014, 2015 and 2016 designated as A, B, and C respectively.

Locus	Allele Frequencies			Changes in allele frequencies		
	2014 (A)	2015 (B)	2016 (C)	B-A	C-A	C-B
BMd20	0.143	0.099	0.09	-0.044	-0.053	-0.009
BM211	0.083	0.083	0.071	0	-0.012	-0.012
C119	0.031	0.05	0.039	0.019	0.008	-0.011
BMd07	0.083	0.143	0.25	0.06	0.167	0.107
BMd53	0.333	0.25	0.333	-0.083	0	0.083
PV-BR25	0.333	0.3	0.225	-0.033	-0.108	-0.075
BMd28	0.048	0.036	0.049	-0.012	0.001	0.013
BMd01	0.5	0.5	0.333	0	-0.167	-0.167
BM137	0.143	0.091	0.091	-0.052	-0.052	0
BMd18	0.143	0.143	0.143	0	0	0
Pv-atgc002	0.167	0.2	0.2	0.033	0.033	0
BMd03	0.143	0.143	0.143	0	0	0
Pv-ag003	0.333	0.333	0.333	0	0	0
Pv-cttt001	0.099	0.143	0.1	0.044	0.001	-0.043
BM33	0.111	0.199	0.091	0.088	-0.02	-0.108
Pv-tttc001	0.333	0.2	0.267	-0.133	-0.066	0.067
BMd32	0.333	0.5	0.333	0.167	0	-0.167
Pv-at007	0.15	0.067	0.067	-0.083	-0.083	0
Pv-gat001	0.099	0.089	0.067	-0.01	-0.032	-0.022
Pv-gaat002	0.5	0.333	0.25	-0.167	-0.25	-0.083
Mean	**0.205**	**0.195**	**0.174**	**-0.01**	**-0.031**	**-0.021**
Std Err	**0.032**	**0.031**	**0.024**	**0.017**	**0.018**	**0.016**
Coeff of var	**69.914**	**70.118**	**61.958**	**-724.574**	**-260.733**	**-333.827**

Throughout this estimation of the allelic frequency and estimate of gene flow, the discriminatory power of the microsatellite markers was revealed by their variation in the values over the growing seasons (Table 7.1 – 7.3). In Annex 3, the genetic changes over the landraces by growing season were reported and it remained fairly constant for Mbala mixture and Solwezi, and changed fairly well although not significantly for Lusaka yellow and Lundazi. SSR markers such as BMd07, BMd53, BMd28, BM33, Pv-at007 and BM137 amongst others. Annex 4 further showed that the genetic composition of Lusaka yellow shifted from Andean bean dominated to Andean and Mesoamerican bean mixture from 2014 to 2016 as seen by the specific allele sizes based on the reference genotypes' allele sizes. Lundazi beans had a composition of both Andean and Mesoamerican beans from the start of 2014 although the proportion of Mesoamerican beans increased from 2014 to 2015, and again decreased from 2015 to 2016 (Table 7.4 and Figure 7.1).

Table 7.3 Estimated gene flow (Nm) and their changes over the three growing seasons of 2014, 2015 and 2016 designated as A, B, and C respectively.

Locus	Estimated gene flow (Nm)			Changes in the estimated gene flow (Nm)		
	2014 (A)	2015 (B)	2016 (C)	B-A	C-A	C-B
BMd20	0.038	0.131	0.873	0.093	0.835	0.742
BM211	0.538	0.887	0.901	0.349	0.363	0.014
C119	0.595	0.056	0.094	-0.539	-0.501	0.038
BMd07	0.956	0.412	0.309	-0.544	-0.647	-0.103
BMd53	0.437	0.255	0.112	-0.182	-0.324	-0.142
PV-BR25	0.437	0.096	0.067	-0.341	-0.370	-0.029
BMd28	0.634	0.505	0.339	-0.129	-0.295	-0.166
BMd01	0.071	0.055	0.052	-0.017	-0.019	-0.003
BMd137	0.434	0.475	0.285	0.041	-0.149	-0.189
BMd18	0.697	0.183	0.171	-0.513	-0.525	-0.012
Pv-atgc002	0.469	0.206	0.133	-0.263	-0.337	-0.074
BMd03	0.513	0.047	0.203	-0.466	-0.310	0.156
Pv-ag003	0.560	0.147	0.128	-0.413	-0.432	-0.019
Pv-cttt01	0.711	0.200	0.126	-0.511	-0.585	-0.074
BM33	0.377	0.170	0.183	-0.207	-0.194	0.013
Pv-tttc001	0.784	0.069	0.017	-0.715	-0.766	-0.051
BMd32	0.115	0.627	0.001	0.512	-0.114	-0.626
Pv-at007	0.128	0.073	0.057	-0.055	-0.071	-0.016
Pv-gat001	0.641	0.132	0.090	-0.510	-0.552	-0.042
Pv-gaat002	0.023	0.157	0.235	0.134	0.212	0.078
Mean	**0.458**	**0.244**	**0.181**	**-0.214**	**-0.277**	**-0.063**
Std Error	**0.059**	**0.050**	**0.043**	**-0.009**	**-0.016**	**-0.007**
Coeff of var	**57.75**	**91.9**	**112.8**	**-153.61**	**-158.08**	**-941.11**

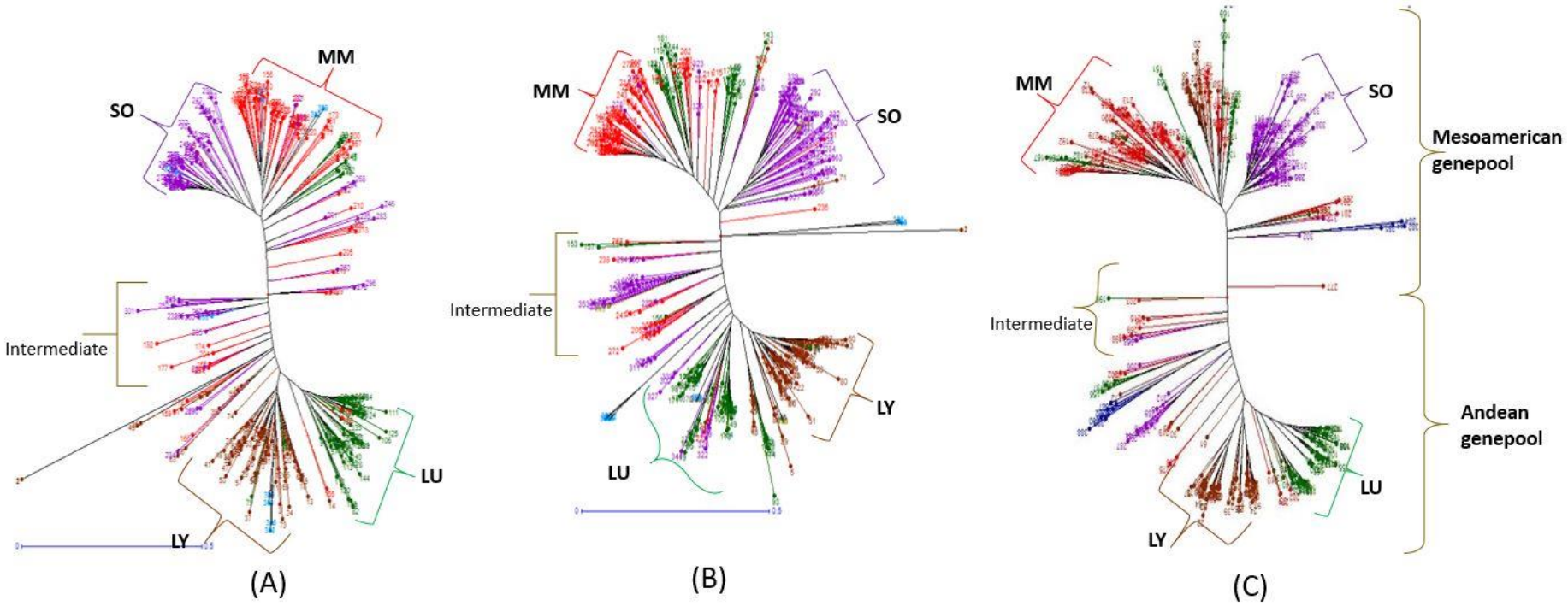


Figure 7.1 Population structure of the Zambian common bean landraces (Lusaka yellow – LY, Lundazi – LU, Mbala mixture – MM, and Solwezi - SO) over the three growing seasons: 2014 (A) had 320 individuals, 2014 (B) had 392 individuals, and 2016 (C) had 392 individuals of all the landraces and CIAT reference lines studied. The number of sub-clusters for Lundazi (Green colour) increased from 2014 to 2016, and some few individuals Lusaka yellow appeared in the Mesoamerican genepool in 2016 for the first time. The light and dark blue colours represent CIAT reference lines.

Analysis of molecular variance showed that the inbreeding co-efficient (Fis) values were high and increased from 2014 (0.633) to 2016 (0.756), with very significant probability ($p < 0.05$) as in table 7.5.

Table 7.4 Changes in Inbreeding coefficient and probability over the three growing seasons

F-Statistics	2014		2015		2016	
	Value	Probability	Value	Probability	Value	Probability
Fis*	0.633	0.001	0.705	0.001	0.756	0.001
Fit**	0.727	0.001	0.809	0.001	0.833	0.001

* Fis = AI / (WI + AI), **Fit = (AI + AP) / (WI + AI + AP) = (AI + AP) / TOT, where AP = Estimate of variation among populations, AI = Estimate of variation among individuals, WI = Estimate of variation within individuals. Both Fis and Fit are used to measure the rate of outcrossing interchangeably.

Partitioning the variation observed for each growing season shows that these variations follow a similar trend of being highest among individuals, followed by among populations, and lowest in within individuals for all the growing seasons (Figure 7.2). This is a similar trend reported on the overall source of variations at molecular level under chapter 5.

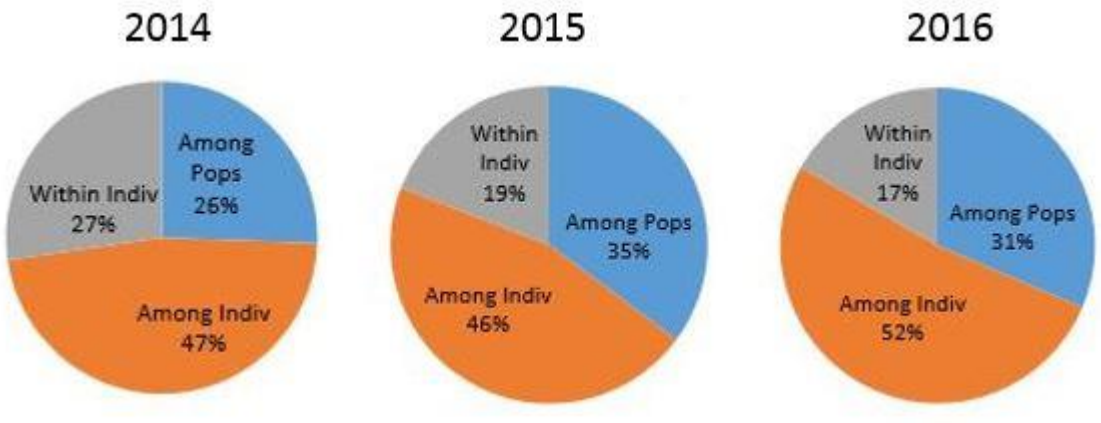


Figure 7.2 Molecular Variance and Changes over the three seasons (2014-16) showing the major contributors to the total variance being observed. Total variance is greatest among individuals, followed by among populations, and lowest in within individuals.

7.2.3 Agro-morphological changes over the growing seasons (2014-2016)

In assessing the agro-morphological changes over the growing seasons, we consider both qualitative (flower and seed colour) and quantitative (100 seed weight) data. Additionally, the seed lengths and widths of the seed produced from Zambia (parental) and the seed produced from Bath (offspring) were compared by way of paired t test, and two way ANNOVA in R. Shapiro test was performed on 100-seed weight, sees lengths and widths of seeds produced from the two locations and confirmed that the data is normally distributed and thus the subsequent analyses followed. The results of one way ANOVA based 100-seed weight and 10 populations of landraces ($df = 9$; $p = 0.0000025$), parental length ($df = 9$; $p < 2x10^{-16}$), offspring length ($df = 9$; $p < 2x10^{-16}$), parental width ($df = 9$, $p = 8.39x10^{-07}$) and offspring width ($df = 9$, $p = 7.74x10^{-05}$) showed that there are significantly differences among the landraces produced over the two locations of Zambia and Bath. A two way ANOVA produced similar results too, except that their interactions were not significantly different (Table 7.5).

Generally, a paired t-test between seed length and width for the parental seeds (produced in Zambia) and the offspring seeds (produced in Bath) showed that there is no significant difference ($p < 0.05$) for landrace in terms of lengths ($df = 239$, $p = 0.004324$) and widths ($df = 239$, $p < 2.2x10^{-16}$). However, a paired t-test for each landrace indicated that there is significant difference between parental and offspring for lengths and widths in the landraces of Lusaka yellow, Lundazi and Mbala mixture, except the landrace of Solwezi for seed length and CIAT G9794 for both seed length and width (Table 7.5 and 7.6). A further Tukey multiple comparisons of mean difference between the landraces was performed in R package and it confirmed that these significant differences vary among the landraces (Annex 5 and 6 for one way and two way ANOVA respectively).

Based on 100 seed weight, the seeds produced from Bath were heavier than the seed produced from Zambia, although they follow similar trends on average for the growing seasons, that is, heavy in 2014 (34.33 and 31.91), lowers in 2015 (31.58 and 26.84), and become heavier again for 2016 (35.16 and 31.72) for Bath and Zambia locations respectively. Of the landraces, 100-seed weight for Lundazi and Solwezi follow a similar trends for the two locations over the three growing seasons, but Lusaka yellow and Mbala mixture did not (Figure 7.3). Generally, for seed lengths, there was more variation in the seeds produced from Bath than from Zambia except for Lusaka yellow and CIAT G9794, whereas for seed widths there was more variation in the seeds produced from Zambia than from Bath except for Lusaka yellow and CIAT G9794.

Genetic correlations, which is a measure of genetic contribution from the parents to their offspring (Griffiths *et al*., 2000), showed both positive values for seed length and width for all the landraces and CIAT lines except for Lundazi (seed length) and Mbala mixture (seed width) that produced negative values. The positive values ranged from 0.0537 in CIAT G9794 to 0.7626 in Solwezi showing difference in genetic contributions to the offspring by these landraces as challenged by environmental conditions (Table 7.5 and 7.6). Additionally, irrespective of the landraces, seed length is more linearly correlated than seed width between the parents (seeds from Zambia) and offsprings (and seeds produced from Bath) as presented in Figure 7.4.

The number of sub-populations varied significantly ($p < 0.05$) over the three growing seasons for each landrace (Figure 7.5). The growing season of 2015 had the highest number of sub-populations for all the landraces, followed by 2014 and lastly 2016. On average, Mbala mixture and Solwezi landraces (Mesoamerican genepool dominated) had the highest number of sub-populations compared to Lusaka yellow and Lundazi (Andean genepool dominated).

Table 7. 5 Summary from the two way ANOVA of parental and offspring seed length and width performed in R package

Two way ANOVA	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Parental Length	1	506.4	384.8	69	<2e-16 ***
Landrace	9	119.3	13.3	10.074	3.4e-10 ***
PL:Landrace	9	8.8	1.0	0.746	0.666
Residuals	80	105.3	1.3		
Offspring Length	1	458.9	458.9	350.734	<2e-16 ***
Landrace	9	97.0	10.8	8.235	1.45e-08 ***
OL:Landrace	9	9.9	1.1	0.837	0.584
Residuals	80	104.7	1.3		
Parental Width	1	2.692	2.692	14.396	2.87e-04 ***
Landrace	9	24.703	2.7448	14.681	9.71e-14 ***
PW:Landrace	9	1.798	0.1997	1.068	0.395163
Residuals	80	14.957	0.1870		
Offspring Width	1	2.483	2.4833	8.638	0.0043 **
Landrace	9	13.322	1.4802	5.149	1.69e-05 ***
OW:Landrace	9	1.929	0.2144	0.746	0.6661
Residuals	80	22.999	0.2875		

**significant at p<0.01, and ***significant at p<0.001.

Table 7.6 Descriptive and statistical analyses for seed variations across the landraces brought from Zambia as Parental (P), and the seeds grown in Bath as Offspring (O) using length (L) and width (W).

Landrace / CIAT LINE	Parameters	PL	OL	PW	OW
Lusaka Yellow	Mean	11.196	11.63	7.189	7.536
	Phenotypic variance	1.894938	1.543867	0.2033656	0.1254489
	Coeff. var	12.29517	10.68379	6.272927	4.699942
	Paired t-test (P=0.05)	0.4688		0.07171	
	Genetic corr (r)	0.2071		-0.23787	
Lundazi	Mean	14.528	14.967	7.138	7.221
	Phenotypic variance	2.12104	3.02029	0.1943956	0.5062544
	Coeff. var	10.02464	11.61153	6.176844	9.853422
	ANOVA (P=0.05)	0.548		0.7667	
	Genetic corr (r)	-0.23912		0.41249	
Mbala Mixture	Mean	11.857	12.29	6.987	7.331
	Phenotypic variance	3.247001	3.838	0.5872233	0.4455656
	Coeff. var	15.1973	15.94045	10.96758	9.105265
	Paired t-test (P=0.05)	0.6132		0.2986	
	Genetic corr (r)	0.4735		-0.325	
Solwezi	Mean	11.203	12.531	7.18	7.595
	Phenotypic variance	0.966423	2.072721	0.4363556	0.3465611
	Coeff. var	8.775045	11.48907	9.200169	7.751079
	Paired t-test (P=0.05)	0.02693*		0.1553	
	Genetic corr (r)	**0.7626**		0.41407	
CIAT G4494	Mean	16.475	16.582	7.867	7.978
	Phenotypic variance	0.775516	1.689351	0.1089344	0.07930667
	Coeff. var	5.345276	7.838321	4.195401	3.529887
	Paired t-test (P=0.05)	0.8318		0.4291	
	Genetic corr (r)	0.20573		0.06663	
CIAT G9794	Mean	9.672	8.331	7.368	6.235
	Phenotypic variance	0.293151	0.1404989	0.1511067	0.08878333
	Coeff. var	5.597956	4.499242	5.275847	4.778915
	Paired t-test (P=0.05)	0.000004639*		0.0000008556*	
	Genetic corr (r)	0.31359		0.05372	

*Significant at P ≤ 0.05, and the bold figure under Solwezi shows greater and significant genetic contribution to seed length.

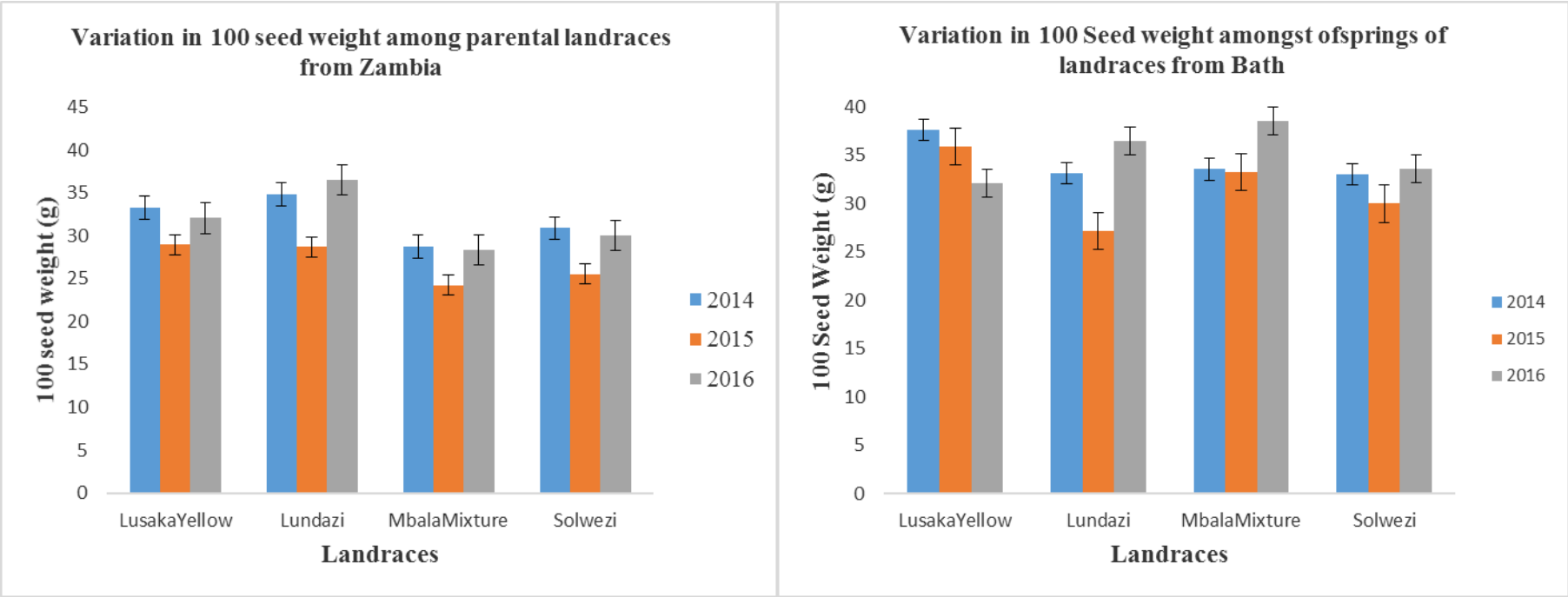


Figure 7.3 Variations in the 100 seed weight between the original seeds received from Zambia and the seeds produced in the Bath tropical glasshouse for all the landraces over the three years of 2014, 2015, and 2016. No similar trends were observed for all the landraces except for Lundazi for the two locations. Seeds received from Zambia were regarded as parental over those produced at Bath as offspring for the different years.

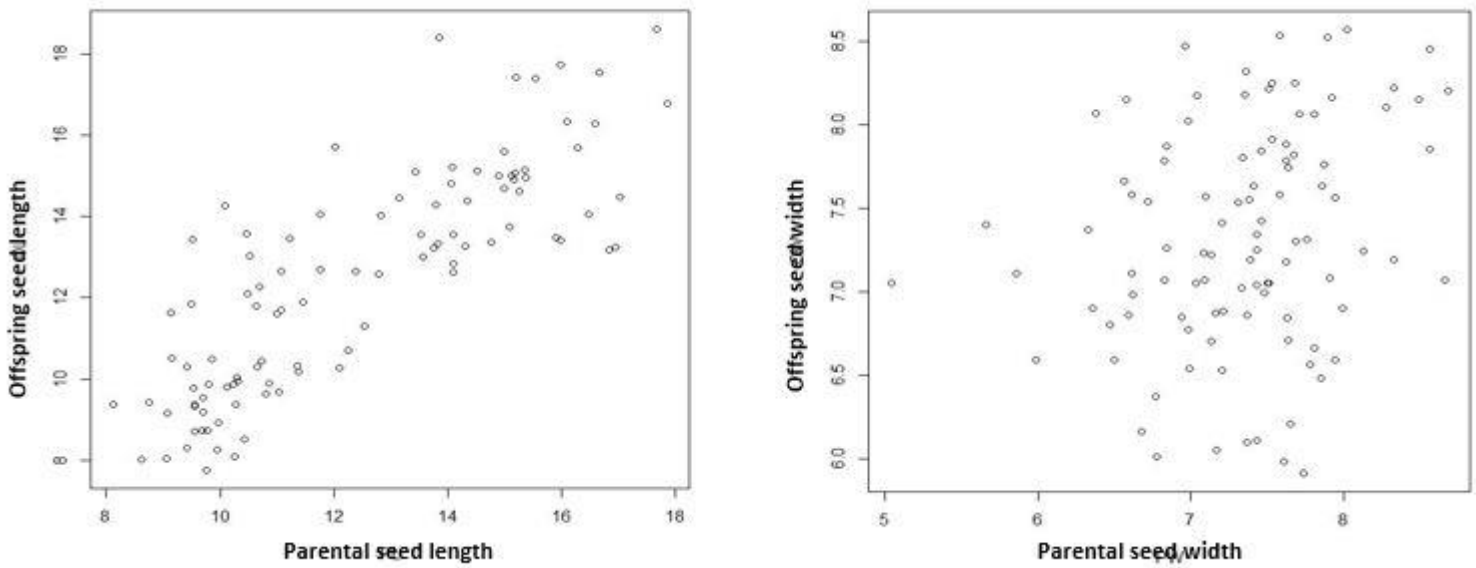


Figure 7.4 Correlations ($p<0.05$, $r = 0.876$) between parental and offspring seed length (left) and seed width (right). A strong linear correlation is observed for seed length (mm) and not for seed width (mm).

Figure 7.5 Agro-morphological variation in the seed types over the three growing seasons for the four landraces. The growing season for each landrace is separated by masking tape boundaries within each landrace.

7.3 Discussion

On average over the three growing seasons, the landraces had a higher genetic diversity (0.421) than the CIAT reference lines (0.259). A close look at the results from the landraces indicated that, there was a decrease in genetic diversity from 2014 (0.436) to 2016 (0.408). The genetic diversity of the Zambian landraces of common bean was lower than those observed by Burle *et al*. (2010) at 0.46 and Okii *et al*. (2014b) at 0.45 for the Brazilian and Ugandan landraces of common beans respectively. However, as explained in chapter 5, several factors including the quantification techniques, types of markers used, population size studied, level of admixture, prevailing growth conditions, and the sampling techniques all play parts in the overall diversity being reported. Particularly the effect of sample size in this current study can be confirmed by looking at the level of genetic diversity reported under chapter 5 when all the individuals over the three years were considered as a single data point (1101 individuals) to the one of this chapter when each of the three years were considered as separate data points. It becomes clear that the more individuals sampled/studied result in higher genetic diversity.

A higher level of diversity observed in the landraces than the CIAT reference lines, and the subsequent decreasing trend from 2014 to 2016 reflects a common 'domestication syndrome' that has been reported by several authors (Gepts, 2002; Burke *et al*., 2002; Gepts and Papa, 2003, 2004; Papa, 2005), and is not unique to this study alone. The previous studies mentioned here noted that, most traits for the domestication syndrome is a result of the action of one or few genes, and that domesticated alleles that are mostly recessive and originate from mutation with a loss of function. Ellstrand *et al*. (1999) demonstrated examples of gene flow between wild and domesticated populations that occur in both directions; from domesticated to wild, and from wild to the domesticated crops. Papa (2005) explained this observation based on population size, that is, at the beginning of agriculture there were more wild plants compared to the domesticated plants, hence gene flow was greatest from the wild to the domesticated crops. This implies that the current agricultural practices which are dominated by domesticated crops, hence gene flow is from domesticated to the wild ones, except for some breeding programmes when particular genes may be sourced from the wild and transferred to domesticated crops. Therefore, the reduction in genetic diversity noted in this present study is a reflection of 'domestication syndrome', is an indication of gene flow from the landraces to the commercial varieties because of their number in the seed composition/admixture but the changes in this diversity over the three growing season was not statistically different.

A decreasing trend in the gene flow rates between these three growing seasons indicate that there is a reasonable amount of gene flow among the sub-populations from one growing season to the other. However, a fact that these rates are not significantly different from each other support the asymmetrical model of gene flow in common bean that affects population structure and not genetic diversity as explained by Papa (2005). Additionally, in chapter 5 and 6 of this thesis, results showed that these landraces exist as admixture comprising of both commercial varieties and landraces with varying levels of seed composition, a factor that is key in asymmetrical gene flow model. Papa and Gepts (2003, 2004) further explained that seed admixture in common bean supports asymmetrical gene flow because pollen dispersal is affected by distance and that farmers' selections of F_1 hybrids affect the total population structure and genetic diversity of the sub-populations involved.

Ferreira *et al*. (2007) studied the effects of distance on the rate of gene flow on common beans where they concluded that rates of gene flow decreases with increasing distance with the highest rate (0.136%) being achieved at 0.5 to 1.0 metres, while at 3.25 metres, the rate was practically zero (0.0045%). It is sufficient to note here that, in their study, they used two pure lines that were planted in rows with the donor variety being planted in the centre surrounded by the recipient variety. In this current study, the admixture is planted together with the different seeds adjacent to each other, or they even could be in the same planting hole, which increases the chances of gene flow between the sub-populations, which may account for the higher rate of gene flow observed in this current study than in the one reported by Ferreira *et al.* (2007). Additionally, we used a one-metre distance was allowed between plots which was still sufficient to allow gene flow between different plots bordering one another as pointed out by Ferreira *et al.* (2007). Therefore, the isolation distances required for growing of certified seeds of common bean was set at 5 metres (Ferreira *et al*., 2007), to maintain genetic purity for these certified seeds.

The fact that allele frequencies and estimated rates of gene flow are not significantly different ($p < 0.05$) from each other over the three growing seasons suggest that the molecular changes that are occurring in these landraces require a considerable long period of time for their effects to be felt in changing the genetic diversity and the population structure. This supports farmers' practices in that the F1 hybrids resulting from these outcrossing events are not selected against, but are retained. This is good news for landraces registration because they would maintain their genetic mixture with minimal change over a long period of time. Beebe *et al*. (1997) noted that the low rate of gene flow in common bean results from its autogamous nature that requires

hundreds to thousands of years to affect genetic diversity and population structure of the common. This system is very different for example, from maize/corn, which is an allogamous in nature and has very high rates of gene flow (20 - 40%) that require a shorter period of time to affect its genetic diversity and population structure (Doebley *et al*., 1990; Goggi *et al*., 2007).

The analysis of molecular variance (AMOVA) results showed an increase in the inbreeding coefficient occurred from 2014 (0.633) to 2016 (0.756). This result confirms that the common beans are not 100 percent inbreeding, and that there is more outcrossing when the landraces have just been brought together compared to when they have been grown together in close proximity for long period due to allele frequency homogenisation as explained by Papa and Gepts (2003, 2004) and Papa (2005). AMOVA results further confirm the differences in genetic diversity over the three growing seasons, and go on to partition this diversity to different sources of variation that was greatest among individuals (48.33%), followed by among population (30.67%), and lowest within individual (21.67%) over the three growing seasons of the total genetic variance. This result fits well with the fact that individuals of the different landraces overlap, hence, a higher source of variation among individuals within a landrace than among population and within individuals. Papa and Gepts (2003) found similar results when they analysed the sources of the total variance detected in their study of asymmetric gene flow and differential geographic structure in common beans. They showed that 44.1% was due to within populations, 37.7% was due to among populations and 18.3% within individuals among populations.

Agro-morphologically, changes over the three growing seasons were also noted ranging from flower colour, 100 seed weight, seed length and width, and number of sub-populations for each of the landraces over the growing seasons. By comparing the results from this current study to the brochure developed by ZARI and SHA that describe these landraces entitled "The most popular bean landraces of Zambia – the commandants of the bean markets in Zambia", significant differences were noted. In the brochure, the three dominant flower colours of purple, pinkish, and white are reported with both white and purple for Mbala mixture, whereas for this current study, the dominant flower colours were light pink (pinkish), and white as dominant flower colours. In addition to these, dark pink and yellow flower colours emerged under Mbala mixture and Solwezi landraces shown in table 6.2 in chapter 6 of this thesis. While this result shows a qualitative morphological changes in flower colour, and suggests an introgression amongst individuals of these landraces, it is also important to pay attention to factors that affects flower colours such as pigments from anthocyanins or carotenoids, and other factors such as vacuolar

compounds, pH and metal ions, temperature, co-pigments, sugars, anthocyanin stacking and cell shape (Noda *et al*., 1994; Miller *et al*., 2011). Therefore, these colour changes in flower among these landraces cannot be used to confirm changes at genetic level resulting from introgression since the two locations were significantly different from each other.

The variation in 100 seed weight over the three growing season followed a similar trend in the rainfall pattern described in Chapter 4 implying that soil moisture content and nutrients were the main sources of variation for the seed weight. Drought has been reported to affect common bean production in Latin America, and East and Southern Africa (Darkwa *et al*., 2016; Heinemann *et al*., 2016; Mathobo *et al*., 2017) affecting yields, seed size, 100 seed weight, days to flowering and days to maturity (Teran and Singh, 2002). Sources of drought tolerance in domesticated common bean have been identified mainly from the races Durango, Mesoamerica, and Jalisco all of which are from the Mesoamerican genepool (Teran and Singh, 2002; Porch *et al*., 2009; Beebe *et al*., 2013), as well as in wild common beans (Cortes *et al*., 2013). It is worth recalling at this point that, in chapters 5 and 6 of this thesis, it was shown that the Zambian landraces are composed of both Andean and Mesoamerican genepools, although it tends to be dominated by the Mesoamerican. Therefore, the decrease in 100 seed weight from 2014 to 2015 and an increase from 2015 to 2016 growing seasons could be a direct response of the Mesoamerican genepool domination amongst these landraces that have been shown to have a high plasticity in yields and other attributes over both periods water shortage and adequate water (Teran and Singh, 2002; Porch *et al*., 2009). This observation fits well with the results in this current study, in that developmental plasticity was the main reason behind the difference in performance of the landraces under the three growing seasons. During the period of soil moisture stress the small seeded beans predominate, and when conditions return to normal, both seeds sizes are maintained, which followed consistently between 100 seed weight and rainfall patterns over the three growing seasons.

Plants, including common bean, have shown three mechanisms of response to drought: drought escape, drought avoidance, and drought tolerance (Beebe *et al*., 2013). These mechanisms are manifested differently by different varieties of common beans with different levels of responses, including rapid phenological development, development plasticity, and remobilisation of photosynthates to the grains, leaf movement /rolling, osmotic adjustments, and the stem reserving utilisation for grain filling (Blum, 2005; Castonguay and Markhart, 1991; Klaedtke *et al*., 2012; Beebe *et al*., 2013). Further experiments to understand these mechanisms in common beans involved grafting (Markhart, 1985; White and Castillo, 1992). These authors showed that root

architecture determines the leaf water potential and promote hydraulic conductivity within the plants. Acosta-Gallegos and White (1995) considered phenotypic plasticity as another mechanism that increases performance under drought stress. Lizana *et al*. (2006) showed that plasticity occurs at the biochemical and cellular level when plants are exposed to drought stress, in terms of stomatal conductance, photosynthetic rate, abscisic acid synthesis, and resistance to photo inhibition. Therefore, the plasticity being reported in this current study is directly linked to the genetic composition of the seed admixture, and can change both genetic diversity and population structure of admixture in question depending on sampling procedure and if it goes on for over a long period of time.

The result which showed that the seeds harvested from Bath were heavier than their counterparts harvested from Zambia did not come as a surprise. The key factors that may be used to explain these variations are soil fertility levels and soil water contents of these locations. The seeds harvested from Bath were planted on a mixture of fine, sandy and coarse composts in the ratio of 2:1:2 respectively. This mixture was again supplemented with a slow nutrient releasing fertilizer called Osmocote Extract (Standard 12-14M, ICL, UK) at a rate of 5 grams for each 5 litre pot and the plants were well watered thoroughly during the growth periods. Osmocote Extract is a compound NPK (Mg) fertilizer with micro nutrients (boron, manganese, copper, zinc, iron, and molybdenum) included to provide nutrients over the entire growth cycle of the plants especially form ported plants. Therefore the seeds harvested from Bath grew under optimum growth conditions compared to their Zambian counterparts that were subjected varying levels of environmental stresses and selection pressure, and hence the normal physiological processes of grain filling, and pod development were much more affected in Zambia than in Bath as explained earlier under water deficit condition. Besides soil water content and soil nutrient as limitations to yields and 100 seed weight in common bean, Legesse *et al*. (2013) showed that Soil pH had a significant differences on the growth, maturity and yields of common beans varieties in Western Ethiopia. On average, a yield reduction of 26% was reported by Legesse and his colleagues. Considering the low levels of soil pH reported under chapter 4 of this thesis, it suggests that soil pH could have affected the 100 seed weight of the seeds harvested from Zambia. Soil pH affects nutrients availability in the soil, promotes aluminium toxicity, and reduces on the cation exchange capacity of the soils all of which have a direct effects on the growth and development of the plants.

The values of genetic correlation for all the landraces between the parental (seeds from Zambia) and offspring (for seeds from Bath) length were positive for Lusaka yellow, Mbala mixture, Solwezi, CIAT G4494 and CIAT G9794 except for Lundazi ranging from -0.239 in Lundazi to 0.763 in Solwezi (table 7.6). In terms of seed width these values were positive for Lundazi, Solwezi, and CIAT G4994 and negative in Lusaka yellow and Mbala mixture, ranging from -0.325 in Mbala mixture to 0.414. As Griffiths *et al*. (2000) explained, these values range from 0 to 1, and figure that tend towards 1 shows greater genetic contribution from parents to offsprings. From the above, we can see that the genetic contribution from parents to offsprings was greatest in seed length than seed width. The high value obtain in Solwezi for both seed length and width can be explained by the high number of commercial varieties in the seed mixture of Solwezi landrace. This could be the same reason for the results that a paired t test found no significant difference ($p < 0.05$) in the seed length between the parental and offspring in Solwezi. The significance difference in other landraces for both seed length and width suggests that there is differences in response to both environmental and or genetic factors by these landraces.

The sub-populations under each landrace (Figure 7.3) followed a similar pattern as explained under 100 seed weight above. More sub-populations were detected in 2015, which had the lowest amount of rainfall on average, giving a reliable clue on the Mesoamerican dominance of the Zambian landraces of common bean. Mbala mixture and Solwezi that been shown to have more individuals under Mesoamerican and higher number of commercial varieties showed the highest number of sub-population in both growing seasons. This could partly be explained by phenotypic plasticity of the Mesoamerican genepool to perform both in deficit water areas and abundant water areas (Teran and Singh, 2002; Porch *et al*., 2009; Beebe *et al*., 2013), and the high rate of crossing between the individuals of the commercial varieties and landrace individuals within these landraces (Papa and Gepts, 2003 and 2004; Papa 2005; Ferreira *et al*., 2007). This result further suggest that although commercial varieties could come from both genepools, under stressed water conditions, the influence of Mesoamerican genepool is always greatest.

In summary, there were both molecular and morphological changes occurring to these common bean landraces over the three growing seasons, but these changes were not significant ($p < 0.05$, for t-tests, one and two way ANONA) enough to change the genetic make and or seed composition of these landraces. Therefore, for a significant change to occur, there is need for a considerable long period of time not just three growing seasons. This give the first insight of the changes in common bean landraces over time and could prove very useful when it comes to seed

conservation and registration for these landraces. Combining the results from this chapter and earlier Chapters (4-6) discussed, it can therefore be summarised that, genetic diversity and population structure of landraces of common bean from Zambia in an interplay of many factors including: farming system, rainfall pattern, soil nutrients (fertility), soil pH, seed admixture, porous borders, aims and objectives of the different stakeholders involved along the bean value chain (breeders and seed companies inclusive), and the original seed introductions from the bean genepools. However, phenotypic plasticity functions to maintain these high levels of diversity among the different environmental challenges, together with farmers' selection pressure.

Chapter 8

Macro and Micro Element Concentrations and their Diversity of the Common Bean Landraces from Zambia

8.1 Introduction

Common bean is grown for its fresh leaves and pods as vegetables, and dry grains with most nutritional properties linked to their high protein content, carbohydrate, vitamins and mineral content as presented in Table 2.1 (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010; Mukamuhirwa *et al*., 2012; Petry *et al*., 2015; Chavez-Mendoza and Sanchez, 2017) in Latin America, Eastern and Sub Saharan Africa and South East Asia. Despite the contribution from common bean and other food staples, the prevalence of micronutrient deficiencies (MND) is still high in developing countries and are caused mainly by lack of essential vitamins (Vitamin A) and minerals (Iron and Zinc) (Ronah *et al*., 2017). WHO, (2009) and Darnton-Hill *et al*. (2005) reported that iron deficiency anaemia (IDA) is the most prevalent micronutrient condition globally, with 65.5% of pre-school children suffering from anaemia, while 45.7% - 48.2% of women of reproductive age suffer from IDA, and that Vitamin A deficiency (VAD) affects 190 million children under the age of five. Welch and Graham (2004) presented a total of 49 known essential nutrients needed to sustain human life together with some of their average energy allowance (AEA), recommended dietary allowance (RDA), estimated safe and adequate daily dietary intakes (ESADDI), and minimum requirement (MR). Welch and Graham (2004) further noted that, in our pursuit of increasing the availability of these minerals, there are both enhancing substances such as ascorbic acid and S-containing amino acids that promote micronutrient bioavailability and decreasing antinutrient substances such as phytate and polyphenolics that inhibit micronutrient bioavailability, hence the need for caution.

The roles of these minerals in the human diet have been elaborated upon by many authors (Agarwal *et al*., 2011; Akond *et al*., 2011; Alzahrani *et al*., 2017). For instance, calcium (Ca) and magnesium (Mg) play important roles in the development of bone and structural tissue formation, glucose and protein absorption and metabolism, regulation and dilation of blood vessels and regular heart beat (Agarwal *et al*., 2011; Alzahrani *et al*., 2017). Kosch *et al*. (2001) explained that, the deficiency in Ca and Mg causes weak bone and structural connecting tissue formation, hypertension, and poor glucose absorption and absorption. Akond *et al*. (2011) noted that Ca deficiency is linked to some chronic diseases such as osteoporosis, and that Mg deficiency leads to energy production faltering and insufficient production. Iron (Fe) is a crucial component of

haeme proteins, haemoglobin, and myoglobin required for oxygen transportation and vascular functions (Fraga, 2005), zinc (Zn) serves as a cofactor in many enzymatic reactions (Prassad, 2012), copper (Cu) is a coenzyme and a crucial cofactor in Fe utilisation and is required for cytochrome oxidase redox chemical reaction (Naismith *et al*., 2009), manganese (Mn) is essential for immune system and effective food metabolism in addition to serving as cofactors in many enzymatic reactions (Smith *et al*., 2013).

Due to the prevalence of these MNDs, several approaches have been put in place to mitigate them through supplementation and food fortification. However, supplementation and fortification are cost ineffective and don't reach the rural poor (Welch, 2002), hence the development of bio-fortification. Bio-fortification has been defined differently but the key components are development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology (Nestel *et al*., 2006) or improving the nutritional content of staple crops by breeding varieties that have a high content of the three limiting micronutrients (Vitamin A, Iron, Zinc) or their precursors than conventional ones (Saltzman *et al*., 2013), and a number of advantages associated with it are being advanced (Graham *et al*., 2001; Graham and Welch, 2006). Chavez-Mendoza and Sanchez (2017) and Beebe *et al*. (2000b) observed that iron content values vary between genotypes, and from wild and cultivated beans, although the wild beans had only a narrow advantage in iron content over cultivates one. Specifically, Tryphone and Nchimbi-Msolla (2010) showed that there is are wide genetic variations between genotypes in Iron and Zinc contents in Tanzania, and that these mineral content vary between leaves (310.49 ppm of Fe, and 28.03 ppm of Zn) and seeds (55.01 ppm of Fe, and 31.4 ppm of Zn).

Significant high positive correlations are found among several elements, including iron, zinc, sulphur, manganese, and phosphorus, with iron and zinc had a statistically significant correlation ($r = 0.663$, $p < 0.05$) across different genotypes (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010). The implication of these correlations is that some genetic factors for different minerals are co-segregating and that selection for one element (for example, iron) will in fact result in an increase in other elements (such as zinc). This makes selecting plants with high content in these minerals to be a cheap source of bio-fortification through breeding programs. Several nutritional research challenges have been highlighted during the development and evaluation of these bio-fortified staple crops aimed at fastening the process (Hotz and McClafferty, 2007).

Specific studies that rely on the mineral composition based on germplasm from African origin are still countable as seen in the work of Tryphone and Nchimbi-Msolla, 2010 and Mukamuhirwa *et al*., 2012 for Tanzania and Uganda respectively. This hampers a cheap source of food supplementation through bio-fortification in common beans for East and Southern African countries. The mineral composition and or content of bean germplasm from major producing countries in this regions, including Zambia remain unknown. Further still, how these minerals vary between landraces and commercial varieties has not reported anywhere to the best of our knowledge. This research chapter has been designed to fill this gap by determining the mineral concentrations of the Zambian common bean germplasm by taking into account the seed sources that are commonly used in the participatory bean breeding program that encompasses both local materials (landraces) and commercial varieties of both farmers and breeders respectively.

Therefore the main objective of this chapter is to determine and present the macro and micro element concentrations of the sub-populations within these landraces, and later compares how these vary with the CIAT reference lines and the Zambian commercial varieties. This is aimed at complementing the earlier chapters on the molecular and agro-morphological diversity reported, and further to exploit whether the preferences in some of landraces and their sub populations are due to seed mineral composition. Further still, determination of the mineral composition in this chapter is aimed at fostering bio-fortification by identifying potential parental breeding lines in different sub-populations of these landraces with high Iron and Zinc content.

8.2 Results

8.2.1 Micro and macro nutrients variation in common bean landraces

A significant difference ($p < 0.05$) was recorded for the 100 seed weight (100SW) and micro and macro nutrients in common bean landraces from Zambia, CIAT reference lines and the Zambian commercial varieties (Table 8.1). Sodium was the most variant mineral (with the coefficient of variation = 111.99) while Magnesium was the least variant (with the coefficient of variation = 12.55), and potassium being the most abundant macro elements (with an average of 4786.60±132.51 mg/100g of dry bean seeds) in the common beans germplasm studied. 100SW ranged from 19.76 g in CIAT reference line G5773 to 52.68 g in Mbala mixture (MM1) with an average of 33.89±1.15. Copper ranged from 0.85 mg/100g in Mbala mixture (MM6) to 3.15 mg/100g in a sub-population that had overlapped between Mbala mixture and Solwezi (MS7) with a mean of 2.27±0.07. Iron ranged from 7.0 mg in a sub-population that had overlapped

between Mbala mixture and Solwezi (MS4) to 34.0 mg in a sub-population that had overlapped between Lusaka yellow and Mbala mixture (LY+MM) with a mean of 21.38±0.79. Manganese ranged from 2.25 mg in Solwezi (SO6) to 4.5 mg in a sub-population that had overlapped between Lusaka yellow and Mbala mixture (LY+MM) with a mean of 3.15±0.09. Sodium ranged from 0.05 mg in G14470 CIAT reference material (a commercial variety from Zambia) to 9.3 mg in G4494B CIAT reference line with an average of 1.43±0.21. Zinc varied between 4.0 mg in Sugar bean commercial variety to 12.0 mg in a sub-population that had overlapped between Lusaka yellow and Mbala mixture (LY+MM) with an average of 8.09±0.23. Potassium ranged from 3500 mg in Long White commercial variety to 6100 mg in a sub-population that had overlapped between Mbala mixture and Solwezi (MS3) with an average of 4786.6±132.51. Magnesium varied between 380mg in Katwetwe commercial variety to 690mg in a sub-population that had overlapped between Mbala mixture and Solwezi (MS3) with an average of 563.2±10. Calcium ranged between 250mg in Long White commercial variety to 1275mg in Lundazi landrace with an average of 602.5±29.79.

Table 8.1 Variations in the mineral content: Coper (Cu), Iron (Fe), Manganese (Mn), Sodium (Na), Zinc (Zn), Potassium (K), Magnesium (Mg), and Calcium (Ca) in the landraces of Lusaka yellow (LY), Lundazi (LU), Mbala mixture (MM), and Solwezi (SO), four CIAT Reference Lines, and four Zambian commercial Varieties

Populations	Sub-Pops.	100SW	Cu	Fe	Mn	Na	Zn	K	Mg	Ca
Lusaka Yellow	LY1	27.05	2.40	21.50	3.50	0.60	7.75	3900.00	570.00	650.00
	LY2	29.47	2.50	22.00	3.00	0.70	7.75	5100.00	590.00	950.00
	LY3	34.52	2.20	26.00	3.50	3.40	9.25	4700.00	540.00	800.00
	LY4	48.00	2.55	21.50	3.00	1.00	8.25	4500.00	480.00	375.00
	LY5	31.26	1.50	18.50	3.00	0.65	8.50	4800.00	650.00	675.00
	LY6	24.31	2.95	28.50	4.50	1.20	9.75	5700.00	650.00	775.00
	LY7	29.51	2.80	31.00	4.00	0.70	10.00	5800.00	590.00	650.00
	LY8	26.91	2.10	23.50	4.00	1.70	9.25	5900.00	600.00	950.00
Lundazi	LU1	42.54	2.65	18.50	3.50	1.85	9.00	5000.00	540.00	600.00
	LU2	20.75	3.00	29.50	3.50	0.75	9.50	5200.00	620.00	550.00
	LU3	22.51	2.50	25.50	3.50	5.35	7.00	5300.00	620.00	525.00
	LU4	33.58	2.50	23.00	3.50	0.90	9.00	5200.00	600.00	600.00
	LU5	24.27	2.10	19.50	2.50	1.05	6.00	5000.00	670.00	525.00
	LU6	41.17	2.20	20.50	3.50	4.20	6.75	5000.00	510.00	550.00
	LU7	27.94	2.10	17.50	4.00	2.30	8.75	5200.00	600.00	**1275.00**
	LU8	25.19	1.80	16.50	3.50	0.80	6.50	4400.00	620.00	875.00
Mbala Mixture	MM1	**52.68**	2.40	26.50	2.75	0.80	9.00	5900.00	640.00	725.00
	MM2	30.67	2.10	20.00	2.50	0.50	8.25	5200.00	550.00	600.00
	MM3	38.29	1.75	21.00	2.00	0.45	10.00	4900.00	540.00	650.00
	MM4	31.45	2.45	21.50	3.00	0.75	7.25	5400.00	590.00	900.00
	MM5	26.90	1.80	19.50	3.00	0.60	6.50	3600.00	480.00	575.00
	MM6	50.15	0.85*	9.50	2.00	1.40	5.50	3900.00	460.00	225.00
	MM7	30.44	2.65	25.50	2.50	0.80	8.50	4400.00	480.00	425.00
Solwezi	SO1	51.94	2.55	21.50	2.50	0.90	9.25	4000.00	510.00	575.00
	SO2	32.16	2.65	25.00	3.50	0.70	7.00	4300.00	510.00	500.00
	SO3	40.16	2.90	24.00	3.25	0.90	11.25	4500.00	550.00	400.00
	SO4	20.55	2.50	26.50	3.00	0.90	8.00	4400.00	550.00	450.00
	SO5	30.01	2.15	19.00	2.50	1.20	6.50	5000.00	630.00	450.00
	SO6	37.40	2.30	17.50	2.25*	0.65	7.00	4800.00	510.00	425.00
	SO7	38.50	2.30	23.00	2.50	1.10	9.00	4900.00	510.00	400.00
	SO8	30.07	2.40	26.50	3.50	0.85	8.75	4800.00	590.00	525.00
LY + MM	LYMM	37.51	2.65	**34.00**	**4.50**	1.35	**12.00**	5000.00	540.00	925.00
LU+MM+SO	LMS1	39.21	1.60	16.00	2.50	0.60	6.50	4400.00	510.00	425.00
	LMS2	29.23	2.35	19.50	3.50	0.70	7.00	5400.00	550.00	725.00
MM + SO	MS1	31.83	2.15	17.00	2.50	0.60	6.50	5600.00	650.00	700.00
	MS2	31.57	2.25	21.00	2.75	0.60	8.25	4700.00	600.00	475.00
	MS3	29.41	2.50	27.50	4.00	0.90	9.50	**6100.00**	**690.00**	725.00
	MS4	27.02	2.05	7.00*	2.50	0.90	7.00	6000.00	620.00	650.00
	MS5	37.72	2.75	22.00	3.50	0.70	9.25	5700.00	610.00	950.00
	MS6	38.52	3.10	29.50	3.50	0.80	10.50	5500.00	620.00	875.00
	MS7	49.74	**3.15**	27.00	3.00	0.55	10.50	4400.00	510.00	625.00
	MS8	33.16	2.40	20.50	2.50	0.70	8.00	4700.00	570.00	650.00
CIAT Lines	G5773	19.76*	2.50	25.00	4.00	2.50	9.50	5000.00	540.00	575.00
	G4494B	39.70	2.45	19.50	3.50	**9.30**	7.25	5900.00	680.00	450.00

	G4494C	31.89	2.30	24.50	3.50	0.75	8.25	5500.00	680.00	625.00
	G14470	36.91	2.15	20.00	2.50	0.50*	7.25	430.00	490.00	300.00
Zambian Commercial Lines	Long White	36.35	1.60	14.00	3.00	0.80	6.50	3500.00*	500.00	250.00*
	Kabulangeti	46.16	1.65	13.00	2.75	2.90	5.50	3600.00	430.00	400.00
	Katwetwe	30.81	1.35	11.00	3.00	1.50	6.50	3600.00	380.00*	325.00
	Sugar Bean	37.56	1.10	11.50	3.50	0.75	4.00*	3600.00	440.00	325.00
Mean		**33.89**	**2.27**	**21.38**	**3.15**	**1.34**	**8.09**	**4786.60**	**563.20**	**602.50**
Std Err		**1.15**	**0.07**	**0.79**	**0.09**	**0.21**	**0.23**	**132.51**	**10.00**	**29.79**
Coeff of var		**24.01**	**21.40**	**25.99**	**19.28**	**111.99**	**19.76**	**19.58**	**12.55**	**35.17**

*lowest values, the **bold** values are the highest values for each mineral and 100SW, and all concentrations were reported in mg/100g.

8.2.2. Micro and macro nutrient variation in common bean based on populations

Considering population averages as a whole, Lusaka yellow had three of the highest values for iron, potassium and calcium; Mbala mixture had two of this highest values for manganese and potassium; G5773 from CIAT lines had the highest values for copper and zinc; G4494B from a CIAT line had the highest values for sodium and magnesium; and the commercial variety of Kabulangeti had the highest value for seed weight (Table 8.2). The landraces of Lundazi and Solwezi had neither the lowest nor highest values for the micro and macro nutrients, and the six of the eight lowest values all came from the Zambian commercial varieties while the other two came from the CIAT lines.

Generally, there were high values for these minerals in the landraces followed by CIAT lines and low values were recorded among the Zambian commercial varieties. The Zambian landraces had 5 of the 8 highest values on average for copper, manganese, zinc, potassium, and calcium, CIAT lines had 3 of the 8 for iron, sodium and manganese; and the commercial varieties were high in 100 seed weight only. The Zambian commercial varieties had 7 of the 8 lowest average values for copper, iron, manganese, zinc, potassium, magnesium and calcium in common bean, and the other only lowest value from the landraces was for Sodium.

At population and sub-population levels, analysis of variance showed there were significant different ($p<0.05$) between these mineral contents among populations. Mann-Whitney pairwise significance analysis also showed that there is significant differences ($p<0.05$) between any pair of these micro and macro nutrients in common bean germplasm studied. However, there was no correlation between any of the mineral content and seed size based on 100SW.

8.2.3 Micro and macro nutrient variation in common bean based on seed colour

Where the seeds were arranged according to seed colour, there were no statistical difference ($p<0.05$) between the macro and micro mineral contents for the common bean germplasm. For the results presented here, there was a very minor separation between dark red and maroon, and they could be treated interchangeably. 100SW was highest in green and lowest in yellow; copper was highest in maroon and lowest in white; iron was highest in maroon and lowest in white; manganese was highest in yellow and lowest in pink; sodium was highest in red and lowest in white; zinc was highest in maroon and lowest in white; potassium was highest in red and lowest in purple; magnesium was highest in red and lowest in green; and calcium was highest in red and lowest in green (Table 8.3). Red and maroon took most high values for the mineral concentrations measured, and white look most of lowest values for these minerals, with green colour taking two and purple and pink taking one each of these low values of the mineral concentration. Black, brown, and grey did not take any of the highest or lowest values and were all close to the top values.

Table 8.2 Variation in the mineral content by populatiuons: coper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), potassium (K), magnesium (Mg), and calcium (Ca) among the Zambian Landraces, CIAT reference lines and Zambian Commercial varieties

Origin	Populations	No of sub-pops	100SW	Cu	Fe	Mn	Na	Zn	K	Mg	Ca
Zambian Landraces	Lusaka Yellow	9.00	32.06	2.41	25.17**	3.67	1.26	9.17	5044.44**	578.89	750.00**
	Std Error		2.40	0.14	1.70	0.20	0.30	0.45	18.14	18.14	62.36
	Lundazi	10.00	30.64	2.28	20.60	3.35	1.85	7.60	5010.00	584.00	665.00
	Std Error		2.53	0.41	1.35	0.15	0.52	0.41	110.00	16.94	78.28
	Mbala Mixture	18.00	35.86	2.28	21.36	9.92**	0.76	8.33	5044.44**	567.22	656.94
	Std Error		1.87	0.13	1.56	0.16	0.06	0.41	170.70	15.29	44.96
	Solwezi	18.00	34.90	2.45	21.67	2.96	0.79	8.32	4955.56	571.67	584.72
	Std Error		1.81	0.09	1.26	0.12	0.04	0.36	146.02	13.41	38.73
	Mean for Landraces		**33.36**	**2.36[b]**	**22.20**	**4.98[b]**	**1.17[a]**	**8.36[b]**	**5013.61[b]**	**575.45**	**664.17[b]**
	Standard Error		**2.15**	**0.19**	**1.47**	**0.16**	**0.23**	**0.41**	**111.22**	**15.95**	**56.08**
CIAT Reference Lines	G5773	1.00	19.76*	2.50**	25.00	4.00	2.50	9.50**	5000.00	540.00	575.00
	G4494B	1.00	39.70	2.45	19.50	3.50	9.30**	7.25	5900.00	680.00**	450.00
	G4494C	1.00	31.89	2.30	24.50	3.50	0.75	8.25	5500.00	680.00**	625.00
	G14470	1.00	36.91	2.15	20.00	2.50*	0.50*	7.25	430.00	490.00	300.00
	Mean		**32.07[a]**	**2.35**	**22.25[b]**	**3.38**	**3.26[b]**	**8.06**	**4207.50**	**597.50[b]**	**487.50**
	Standard Error		**4.41**	**0.08**	**1.45**	**0.31**	**2.06**	**0.53**	**1272.55**	**48.71**	**72.53**
Zambian Commercial Varieties	Long White	1.00	36.35	1.60	14.00	3.00	0.80	6.50	3500.00*	500.00	250.00*
	Kabulangeti	1.00	46.16**	1.65	13.00	2.75	2.90	5.50	3600.00	430.00	400.00
	Katwetwe	1.00	30.81	1.35	11.00*	3.00	1.50	6.50	3600.00	380.00*	325.00
	Sugar Bean	1.00	37.56	1.10*	11.50	3.50	0.75	4.00*	3600.00	440.00	325.00
	Mean		**37.72[b]**	**1.43[a]**	**12.38[a]**	**3.06[a]**	**1.49**	**5.63[a]**	**3575.00[a]**	**437.50[a]**	**325.00[a]**
	Standard Error		**3.17**	**0.13**	**0.69**	**0.16**	**0.50**	**0.59**	**25.00**	**24.62**	**30.62**

*lowest population value, **highest population value, [a]lowest mean value, [b]highest mean value, and all concentrations were reported in mg/100g.

Table 8.3 Variation in the mineral contents by seed colours: Zambian landraces, CIAT reference lines and Zambian commercial varieties based on seed colours. The number in bracket after seed colour represents the number of sub-populations in that seed colour.

Seed Colour	Mean/Std Err	100SW	Cu	Fe	Mn	Na	Zn	K	Mg	Ca
Black (5)	Mean	30.84	2.48	23.50	3.00	1.23	8.60	5020.00	582.00	605.00
	Standard Error	3.80	0.17	1.78	0.32	0.32	0.76	131.91	28.53	78.82
Brown (3)	Mean	36.78	2.57	19.00	3.00	0.78	8.83	5200.00	576.67	625.00
	Standard Error	6.75	0.32	6.11	0.29	0.11	1.01	461.88	33.83	14.43
Green (1)	Mean	**48.00**	2.55	21.50	3.00	1.00	8.25	4500.00	480.00*	375.00*
Grey (4)	Mean	37.85	2.13	18.88	2.94	1.19	7.94	4900.00	532.50	681.25
	Standard Error	3.46	0.26	2.02	0.36	0.57	1.03	463.68	37.50	113.36
Maroon (2)	Mean	30.46	**2.95**	**26.75**	3.38	0.83	**10.38**	4850.00	585.00	475.00
	Standard Error	9.71	0.05	2.75	0.13	0.08	0.88	350.00	35.00	75.00
Pink (7)	Mean	38.23	2.15	21.64	2.71*	0.85	8.14	4700.00	557.14	510.71
	Standard Error	4.93	0.22	2.30	0.18	0.12	0.47	263.67	22.64	58.72
Purple (8)	Mean	34.05	1.92	18.50	3.25	1.24	6.75	3978.75*	518.75	503.13
	Standard Error	1.95	0.19	2.09	0.19	0.44	0.53	580.66	34.35	73.25
Red (07)	Mean	33.14	2.35	20.29	3.25	**3.06**	7.68	**5242.86**	**608.57**	621.43
	Standard Error	2.68	0.07	1.25	0.24	1.20	0.38	139.48	24.54	112.78
White (2)	Mean	34.09	1.88*	15.50*	2.75	0.70*	6.50*	4550.00	575.00	475.00
	Standard Error	2.26	0.28	1.50	0.25	0.10	0.00	1050.00	75.00	225.00
Yellow (11)	Mean	29.94*	2.36	24.68	**3.50**	1.13	8.77	4936.36	570.91	**752.27**
	Standard Error	1.13	0.13	1.47	0.20	0.25	0.46	228.14	17.29	52.05

*lowest value, **bold** is the highest value for the mineral content, and all concentrations were reported in mg/100g.

8.2.4 Pairwise correlation analyses of the micro and macro nutrients in common bean

A pairwise correlation analyses produced strong significant ($p<0.05$), positive and non-significant relationships amongst these minerals in common beans landrace (Table 8.4). These strong positive significant relationships were observed between iron and zinc, iron and sodium, iron and calcium, iron and magnesium, sodium and zinc, sodium and potassium, sodium and magnesium, sodium and calcium, zinc and potassium, zinc and magnesium, zinc and calcium, potassium and magnesoim, potassium and calcium, and calcium and magnesium. Technically speaking, the most frequent strong positive correlations were between micro and/or macro elements themselves and less frequent in micro and macro elements relationships.

Table 8.4 Pairwise correlations coefficients for the mineral content of coper (Cu), iron (Fe), manganese (Mn), sodium (Na), zinc (Zn), potassium (K), magnesium (Mg), and calcium (Ca) in common beans.

	Cu	Fe	Mn	Na	Zn	K	Mg	Ca
Cu		0.0223	0.4884	0.1858	0.0370	0.1448	0.0523	0.0390
Fe	0.0223		0.1001	0.6766*	0.8227*	0.3538	0.6221*	0.6566*
Mn	0.4884	0.1001		0.4884	1487	0.3647	0.2083	0.1487
Na	0.1858	0.6766*	0.4884		0.5789*	0.9423*	0.8388*	0.7748*
Zn	0.0370	0.8227*	0.1487	0.5789*		0.5884*	0.8067*	0.8659*
K	0.1448	0.3538	0.3647	0.9423*	0.5884*		0.6969*	0.6516*
Mg	0.0523	0.6221*	0.2083	0.8388*	0.8067*	0.6969*		0.9643*
Ca	0.0390	0.6566*	0.1487	0.7748*	0.8659*	0.6516*	0.9643*	

*Strong positive significant correlations ($p < 0.05$).

8.2.5 Principal component analysis (PCA) and Neighbor joining clustering based on the mineral concentrations in common beans

The multivariate analysis using the principal component analysis (PCoA) (Figures 8.1) and loading values for the different components (Table 8.5) generated the partial distribution of all the sub-populations based on the micro and macro element concentrations. The results indicated that the macro and micro element concentrations can be explained by 8 axes, of which only 4 had significant contribution to the spatial of the sub-populations explaining 70.54 % of the total variability. The first axis (PC1) explained 22.85% of the total variability with elements of Cu, Fe, Na, Zn, K, and Ca being positively significant; the second axis (PC2) explained 18.41% with all the elements being positively significant except K; the third axis (PC3) explained 16.53% with Cu, Fe, Mg, and Ca being positively significant; and the fourth axis (PC4) explained 12.76% with all the elements except Ca and Fe being significant, with the eigenvalues of 1.96, 1.58, 1.42, and 1.10 respectively.

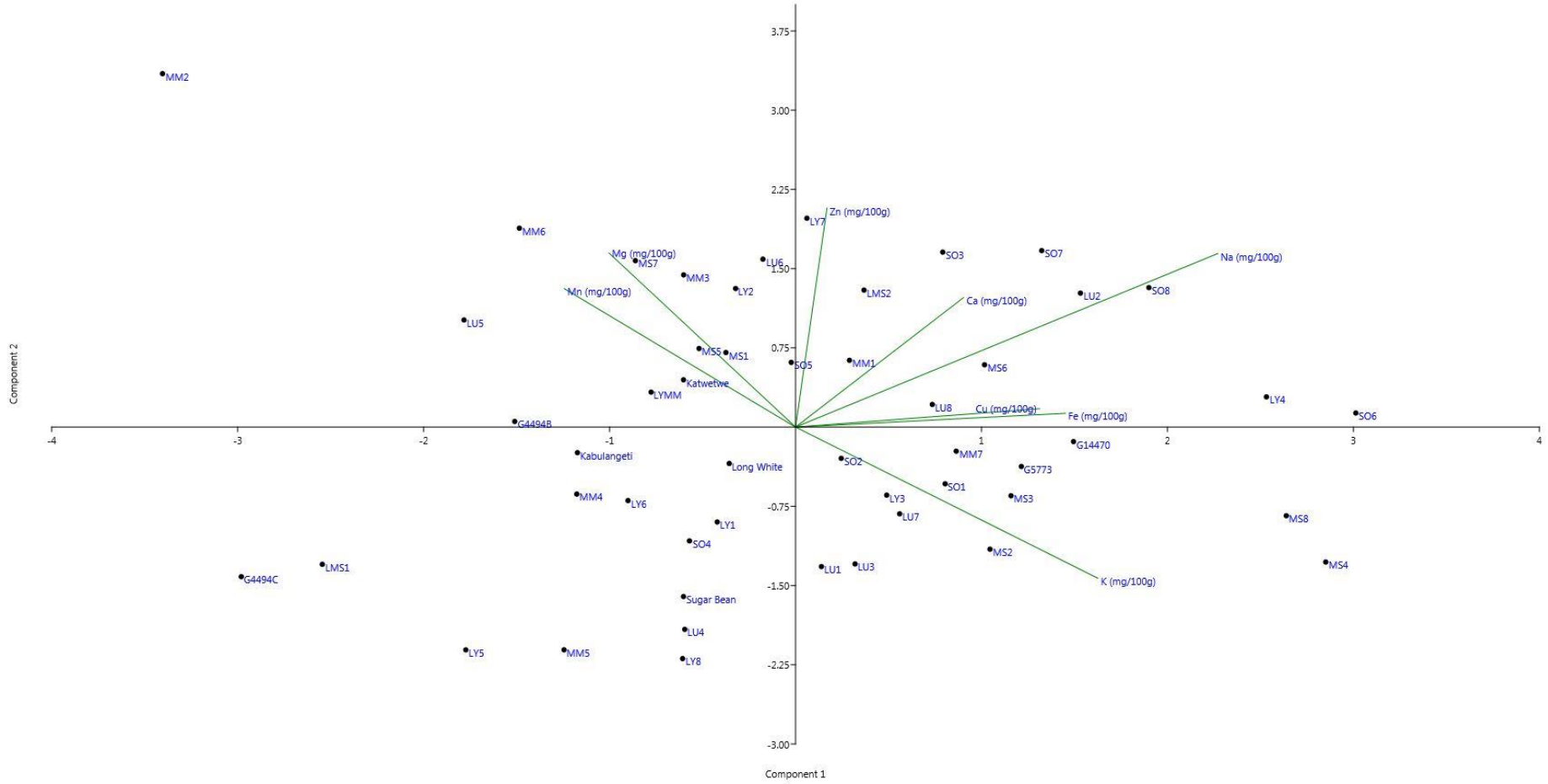


Figure 8.1 Representation of the Euclidean bi-plot by principal component analysis (PCoA) with transformed data for all the variables in the analysis. Legend: Ca – calcium; Cu – Copper; Fe – Iron; K – Potassium; Mg – Magnesium; Mn- Manganese; Na – Sodium; and Zn – Zinc.

Table 8.5 Loading values that show the contribution of mineral content to each principal component (PC) of the PCoA with the common bean landraces of Zambia

Minerals (mg/100g)	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**	**PC8**
Copper	0.3386	0.0450	0.4771	0.1221	0.3058	-0.0058	0.6029	-0.4288
Iron	0.3745	0.0337	0.2826	-0.1864	-0.2445	-0.5448	0.1496	0.6042
Mangnese	-0.3208	0.3381	-0.3657	0.2142	0.4081	-0.0305	0.5001	0.4309
Sodium	0.5858	0.4240	-0.4033	0.0750	0.3092	-0.2437	-0.3466	-0.1838
Zinc	0.0436	0.5348	-0.0870	0.2834	-0.7318	0.1444	-0.2146	-0.1477
Potassium	0.4194	-0.3690	-0.0345	0.6226	-0.0036	0.1445	-0.0497	0.3534
Magnesium	-0.2588	0.4250	0.6108	0.3526	0.1991	-0.0099	-0.4421	0.1408
Calcium	0.2330	0.3162	0.1196	-0.5537	0.0976	0.6709	0.0016	0.2553

8.3 Discussion

The main objective of this chapter was to determine the micro and macro nutrient concentrations in dry seeds of common beans. This study focused on the micro nutrients in common beans as these have been reported to cause the highest global health risks, particularly iron and zinc deficiencies (Welch and Graham, 2004; Darnton-Hill *et al*., 2005; WHO, 2009). Among the macro elements, sodium, calcium, potassium and magnesium were included because of their nutritional importance explained in the human diet as major components of bones and teeth, and proper functioning of muscles and central nervous system (Gouveia *et al*., 2014).

The results from this study showed that, there is are significant differences ($p < 0.05$) in the concentrations of these micro and macro elements in common bean by genotypes. These results agree with previous studies for example, Trphone and Nchimbi-Msolla (2010) reported the variations of iron and zinc between leaves and seeds of Tanzanian common bean genotypes, and plant parts. They showed that for leaves, the average values were 310.49 ppm of Fe, and 28.03 ppm of Zn and for seeds were 55.01 ppm (5.5 mg) of Fe, and 31.4 ppm (3.14 mg) of Zn. Beebe *et al*. (2000b) showed that the average Fe concentration was 5.5 mg/100g and for Zn was 3.5 mg/100g in the core collection of CIAT genotypes. Gouveia *et al*. (2014) worked on the germplasm of Madeira Island of Portugal and showed that on average the mineral concentrations were 1890 mg/100g for K, 150 mg/100g for Mg, 6.01 mg/100g for Fe, 1.01 mg/100g for Cu, 3.01 mg/100g for Zn, and 1.45 mg/100g for Mn. Mahajan *et al*. (2015) studied Fe, Zn and protein contents of common bean genotypes from India and found that on average 1.81 mg/100g was Fe, 0.78 mg/100g was Zn and 20.30% was protein content. Akond *et al*. (2011) studied the mineral concentrations in the 29 genotypes from USA and reported that Zn ranged from 3.4 to 6.4 mg/100g and Fe was from 0.89 to 11.29 mg/100g.

It apparent from the above paragraph that there has been no specific study directed towards the mineral concentrations in the landraces of common bean from any major bean producing regions of the world. This could be the probable reason why the values of the macro and micro elements of this current study are higher than those that had previously reported. However, several factors have been pointed to affect the mineral concentrations of common beans: common bean plant parts (Tryphone and Nchimbi-Msolla, 2010); growing environment and genotypes variations (Moraghan and Grafton, 2001; Akond *et al*., 2011); origin, genotypes, environmental conditions (temperature, soils, and fertilization), growing conditions (Gouveia *et al*., 2014); and the weeding regimes that affect both mineral nutrient uptake and retention in the plant as well as soil moisture for zinc (Glowacka, Klikocka, and Onuch, 2014). When the results of this study are broken down

into landraces, CIAT lines and Zambian commercial lines (Table 8.2) there are higher values on average for Landraces, followed by CIAT lines and lowest in the commercial varieties. It is important to note further here that, the averages of the mineral content of the Zambian commercial varieties fall within most of the values reported earlier. This implies that, the presence of landraces in this study is the reason for the higher average values that this study presents, and it demonstrates how important these landraces can be used in improving nutritional component of the commercial varieties already in production.

This study further presents the mineral composition of the common bean based on seed colours. There was no significance difference ($p < 0.05$) in the mineral content based on seed colour, although there were noticeable differences and consistencies. The higher mineral content values came from mainly maroon (3 values), red (3 values) or yellow (2 values) whereas the lowest values were mainly from white (4 values), green (2 values), pink (1 value) and purple (1 value). Black, brown, and grey seed colours had neither the lowest nor highest values and were consistently high on average. This study shows that the mineral content in common beans do vary considerably, and nutritionally it would be appropriate to begin by balancing the seed colour of common bean for the community that depend entire on common beans. Beebe *et al*. (2000b) had also shown that the tannin content in common bean vary by seed colour. Therefore, the common bean seed colours red, maroon, yellow, brown, black, and grey can be considered for high mineral contents, while white, green, pink, and purple can be considered as low in mineral content to inform our decision making during participatory bean breeding.

Akond *et al*. (2011) studied the mineral compositions of USA genotypes and identified 7 genotypes with high iron and zinc concentrations for each mineral which were recommended to be used as parental for mineral content breeding in USA. In this current study we identified 23 sub-populations with iron concentrations above average (21.38 mg/100g) in Table 8.1: 6 in Lusaka yellow, 3 in Lundazi, 3 in Mbala mixture, 6 in Solwezi, one in an overlap between Lusaka yellow and Mbala mixture, 3 in an overlap between Mbala mixture and Solwezi and 2 in CIAT lines of G5573 and G4494C. This study further identified 26 sub-populations with zinc concentration above average (8.08 mg/100g): 7 in Lusaka yellow, 4 in Lundazi, 4 in Mbala mixture, 4 in Solwezi, one in an overlap between Lusaka yellow and Mbala mixture, 5 in overlap between Mbala mixture and Solwezi, and 2 in CIAT lines of G5573 and G4494C. It is important to point here that, CIAT reference line G4494 had two plant types: a dwarf type with white flowers colour, and a semi climber with a pink flower colour, therefore G4494C refer to the latter

plant type. The overlap between Lusaka yellow and Mbala mixture above is the dominant composition in Lusaka yellow, and had the highest average values for Fe, Zn and Mn with elongate/kidney seed shape with dark blue colour around its helium.

Additional result from this current study showed a non-significant correlations ($p < 0.05$) between mineral contents and 100 seed weight (100SW). This agrees with the findings of Moraghan and Grafton (2001), and Akond *et al.* (2011) when they reported a similar result earlier. Therefore, seed size in mainly an attribute for seed yields as explained by Agung and McDonald, (1998) in Faba beans (*Vicia faba* L) and not for mineral content determination. However, this current study further disagrees with the finding of Beebe *et al*. (2000b) that Fe content tend to be present at higher values in Andean genepool than Mesoamerican genepool. In this current study, we reported Fe in CIAT G5773 – Mesoamerican at 25.0 mg/100g, and in CIAT G4494C – Andean at 24.5 mg/100g that agrees with the results of Akond *et al*. (2011) where they also observe no direct relation with the genepools in relation to the mineral contents for the genotypes from USA.

Positive and negative correlations between the micro and macro elements have been reported in earlier studies (Beebe *et al*., 2000b; Tryphone and Nchimbi-Msolla, 2010; Akond *et al*., 2011; Gouveia *et al*., 2014; Mahajan *et al*., 2015; Alzahran *et al*., 2017). This current study also reported positive and significant correlations ($p < 0.05$) between micro elements: Cu and Zn ($r = 0.699$), Fe and Zn ($r = 0.764$), and Fe and Mn ($r = 0.518$); between macro elements: Ca and Mg ($r = 0.509$), K and Mg ($r = 0.674$), and K and Ca ($r = 0.535$), Na and Ca ($r = 0.720$); and between micro and macro elements: Na and Cu ($r = 0.927$), and Na and Fe ($r = 0.866$). The strong and significant correlations between these micro and macro elements have been explained to mean that the genetic factors for increasing one mineral co-segregate with the genetic factor for increasing the other mineral with which they share a significant correlation (Welch and Graham, 2004; Tryphone and Nchimbi-Msolla, 2010). Therefore, deducing from these results, increasing the contents of iron would increase the content for zinc, manganese, and sodium while increasing the zinc content would increase the content for iron, copper, and sodium. A similar observation was made for macro elements such that increasing the potassium content would increase the content for magnesium and calcium, increasing calcium would increase potassium, sodium and magnesium, and increasing magnesium would increase calcium and potassium.

To explore further the observed correlations among the mineral contents in common bean for breeding purpose, a focus paper by Welch and Graham (2004) observed that, there is significant variability to increase the concentrations of iron and zinc, and that the traits required for the genetic improvement of iron and zinc concentrations are stable across different bean growing environments, although their concentration are affected by GXE interactions. Beebe *et al*. (2000b) had confirmed that, there is an environmental and seasonal stability in the iron concentrations for the CIAT collections. Zemolin *et al*. (2016) studied the genetic parameters of iron and zinc concentrations in Andean bean seeds by looking at their concentrations from crosses between two sets parents (IAC Boreal x Light Red Kidney and Ouro Branco x Light Red Kidney), their F_1 plants, F_1 reciprocals, F_2 plants, F_2 reciprocals, and backcrosses (BC_{11} and BC_{12}). Zemolin and the co-authors concluded that there is no maternal effects, and that the seeds of the F_1 generation will represent fertilisation between the parents in both hybrids combinations. The maternal effect expression for iron and zinc concentration has been linked to the distribution of these minerals to the fractions of the seeds, that is, seed coat or seed embryo depending on the variety and genepool (Possobom *et al*., 2015; Zemolin *et al*., 2016). Therefore, these results mean that there is no time that will be wasted in doing reciprocal crosses with F_1 plants before backcrossing hence allowing segregating populations of common beans with high mineral contents to be generated and deployed within a shorter period of time. Although, Possobom *et al.* (2015) observed a significant expression of the maternal effects in Mesoamerican genepool, they linked this expression of maternal effects to iron accumulation seed coat in Mesoamerica beans or seed embryo in Andean beans. Possobom and his colleagues then suggested that the selection of superior common bean recombinants for iron concentration should begin at F_3 generation if iron accumulates more in the seed coat (Mesoamerican), and at F_2 if the iron accumulates more in the seed embryo (Andean).

Finally on the breeding and inheritance of seed iron and zinc concentrations in common bean, Blair *et al*., (2009c) identified 6 QTLs for zinc and 5 QTLs for iron that are clustered on the upper half of the linkage group B11. Other QTLs for Zn were identified on linkage groups B3, B6, B7 and B9, and B4, B6, B7 and B8 for Fe. This information means that the scientists are getting so close to identify the candidate gene(s) for Fe and Zn concentrations in common bean and will be very useful in the modern era of marker assisted breeding that will shorten the breeding cycle by allowing screening for these minerals at seedling stage of growth for common bean. The PCA distributions and neighbor joining clustering results presented in this chapter support the initial assumption that there is a wide natural variation in the concentrations of macro

and micro elements among these common bean landraces from Zambia that can be exploited for common bean improvement programmes in Zambia, and or provide for a short term material exchange between Zambia and her neighbouring/regional countries.

Chapter 9

General Discussion and Conclusion

9.1 Thesis Summary

This research has demonstrated that there is high genetic diversity and well-structured populations as well as high mineral compositions among these common bean landraces from Zambia. However, when the nutritional qualities were compared among the landraces, Zambian commercial varieties and CIAT reference lines, the landraces had very high values for these nutrients compared to their counterparts. Specifically, this study has identified 26 sub-populations with zinc content higher than the averages and 23 sub-populations with high iron content. The practical implication of this research is that, through breeding, higher yields have been achieved and stabilised; therefore, we need to utilise these landraces to breed for enhanced nutrients in the commercial varieties. The genetic diversity and highly structured populations of these landraces corresponds to the variations observed at the nutritional composition level, hence providing a platform for common bean improvement through using these landraces. Breeding for nutrients using the identified sub-populations in landraces will provide a cheap source for food supplementation whose impacts can directly reach rural resource-poor farming communities. Participatory plant-breeding that incorporates the different stakeholders and/or actors within the common-bean value-chain will be a better breeding approach than the traditional conventional breeding where the researcher develops a variety alone as it will allow breeding of varieties with a focus on the consumers and hence safeguarding the health and environmental concerns of these consumers.

Therefore, this chapter discusses the molecular and agro-morphological methods and their applications in characterisation, identification and diversity assessment of common-bean landraces, gene flow in common beans and its implications. The practical implications of these results in terms of breeding for nutrients enhancement (bio-fortification) and recommendations for future usage of these common bean landraces from Zambia, are then explored. These sub-sections are presented below:

9.2 Molecular and agro-morphological methods in detecting genetic diversity and population structure of common bean landraces

The use of molecular and agro-morphological methods to assess genetic diversity and population structure have been applied to the landraces of several crops: Lentil - *Lens culinaris* M (Fikiru *et al*., 2010); Rice (Bajracharya *et al*., 2006); Wheat (Najaphy *et al*., 2012); Almonds - *Prunus dulcis* (Kadkhodaei *et al*., 2011); and sesame – *Sesamum indicum* L (Pham *et al*., 2011), amongst

others. Similar use of molecular and agro-morphological methods is common in common-beans (Chiorato *et al*., 2006; Asfaw *et al*., 2009; Ligarreto, Gustavo, and Martínez, 2014); however, very few of such studies have been applied to the landraces of common beans grown in Africa. The current study showed that there is a high genetic diversity and population structure among the Zambian landraces based on molecular and agro-morphological methods. This high level of diversity in Zambia can be due to several reasons, including: original introductions, farming systems, rainfall patterns, soil pH that affects nutrient availability, porous borders with neighbouring countries, breeding objectives, market preferences, and the role of the different stakeholders involved in the value chain of common bean. Molecular method identified seven as the appropriate number of sub-populations within these landraces. The value of seven did not come as a surprise since Singh *et al.* (1991b) has indicated that there are seven races among the common beans. Therefore, this result suggests that all the 7 races common bean were introduced in Zambia. However, the difference in the predicted number of sub-populations under molecular and agro-morphological methods could also be attributed to a large difference in the number of individuals studied under the two methods.

Furthermore, the seed size of mainly the medium to larger seeds appeared in Mbala mixture and Solwezi landraces. The subsequent clustering of their individuals under Mesoamerican genepool to a larger extent could suggest two things: i) seed size as an agro-morphological character is no longer a good indication of genepool classification, and ii) the Mesoamerican race of Durango could be the most dominant in Solwezi and Mbala districts of Zambia. Common-bean improvement through breeding has involved hybridization between the two genepools (Brunner and Beaver, 1988; Beebe *et al*., 2008, 2011, 2012 and 2013), and the use of close and wild relatives (Thomas *et al*., 1983; Mejía-Jiménez *et al*., 1994; Keneni *et al*., 2011; Beaver *et al.*, 2012; Porch *et al*., 2013). Surely, seed size in the products of bean improvement could have been affected hence their usefulness in classification of genepools being affected too. Terán and Singh (2002) recorded the average 100SW of Durango to be 34g, and also its plasticity in both low and highland areas in South America. This suggests that the Mesoamerican genepool in Zambia could be dominated by Durango race of common bean. It is worthy noticing here that Mbala and Solwezi areas of Zambia are located in high altitude areas.

Molecular and agro-morphological diversity analyses further revealed that, there is an overlap in the sub-populations of the landraces studied mainly due to the seed admixture practice. Two such common overlaps were either a single landrace being found in different locations, or the different

landraces finding themselves in one location, or both; additionally, the commercial varieties could be found mixed together with these landraces. Such overlaps are key in maintaining the genetic diversity, population structure and nutritional component of these landraces, and could be explained by two factors: a better rate of adoption for the different landraces into one location, or to there being wider environmental adaptations of a single landrace over different locations. Rates of adoption of new varieties in common beans have been explained by many factors, including farmers' indigenous knowledge, farmers' limited access to seed coupled with a failure to promote the variety, and low or fluctuating market demand, yield attributes (David *et al*., 2002), household characteristics, and sex and income status of the farmers (Ronner *et al*., 2017). Yokouchi *et al*. (2016) had also reported similar reasons for the adoption for new rice for Africa (NERICA) rice varieties, specifically pointing out that male farmers affect adoption because of their dominance in land acquisition compared to female farmers, and that information flow (seed and market) and seed availability were other crucial factors that affect the rate of adoption of a new varieties of NERICA rice.

9.3 Molecular and morphological changes over the three growing seasons

Molecular and morphological changes were noticed over the three growing seasons among the common-bean landraces, although these changes were not significantly different. Therefore, combining the high levels genetic diversity reported under Chapters 5 and 6, and the high rate of gene flow presented in Chapter 7, the landraces of common bean present an opportunity to be used in genetic conservation, as well as in breeding of new varieties of common beans that are well adapted to the different agro-ecologies of Zambia, including with enhanced mineral content in their seeds (Beebe *et al*., 2000b; Blair *et al*., 2009c; Possobom *et al.*, 2015). The detection of no significant differences in genetic diversity and population structure between the growing seasons at molecular and morphological levels, suggests sufficient stability of the genetic mixture to enable landrace registration, production and promotion by the communities that depend on them. Also, considering the asymmetrical model of gene flow between landraces and commercial varieties (Papa and Gepts, 2003; Papa and Gepts, 2004; Gepts and Papa, 2004; Papa, 2005), landraces can be very useful in improving both genetic diversity and nutritional status of commercial varieties when they are grown as seed admixture and the resulting F_1 seeds are not selected against. However, for this this to practically work out, it requires the knowledge of correct distance between plants and population size of the seed admixture to be planted in the different plots to be taken into consideration (Ferreira *et al*., 2007).

9.4 Mineral concentration in common bean landraces

This study detected highly significant differences in the micro- and macro-nutrients between the sub-populations studied. The overlap between the different sub-populations in terms of high mineral concentrations was found between Lusaka yellow and Mbala mixture, and between Mbala mixture and Solwezi, for iron, zinc, manganese, potassium, and magnesium. This suggests that seed mineral-content could be a factor that affects the culinary qualities of common bean, and hence their rate of adoption. It is also not surprising that the yellow seed colour is the most dominant seeds among the locally preferred seed types in the Zambian common bean market as discussed in Chapter 4. Determination of nutritional/mineral composition of any food crop, including common bean, provide the very first necessary information needed for bio-fortification. These nutritional/mineral contents are affected by the soil characteristics, origin of the crops, and management options, and vary between wild, weedy, landraces, and commercial varieties (Cakmak *et al*., 2000). Seed size and colour do not present noticeable differences in terms of mineral concentrations in common bean although lower values tend to be associated with white seed colour. This information is very important at a family or institutional level as there is no need to develop preferences for a particular seed colour for consumption as nutrients availability is not guaranteed to be correlated with a single colour.

9.5 Bio-fortification to increase the minerals content in staple food crops

The use of plant breeding for bio-fortification as an approach to increase the mineral content in staple food crops is not unique to common beans. Several efforts have been made in other crops ranging from the identification of sources of variation between genotypes, study on inheritance, QTL mapping, and genome-wide association (GWAS) mappings to gene discoveries, taking both conventional and biotechnological approaches. For instance, in cassava, there have been molecular characterization of cassava with yellow-orange roots for beta-carotene improvement (Ferreria *et al*., 2008), studies on the inheritance of beta-carotene (Akinwale *et al*., 2010), GWAS mapping of provitamin A carotenoid content (Esuma *et al*., 2016), and discovery a phytoene synthase gene that drives provitamin A accumulation in cassava roots (Welsch *et al*., 2010). In rice and maize there have been deliberately two approaches running concurrently, that is, the Golden rice and protein quality maize approach that looks at increasing the beta-carotenoid (Maziya-Dixon *et al*., 2000; Paine *et al*., 2005; Tang *et al*., 2009), and mineral approach focusing mainly on increasing the concentration of iron and zinc in the grains of these crops (Yang *et al*., 1998; Bänziger and Long, 2000; Maziya-Dixon *et al*., 2000; Masuda *et al*., 2009; Prasanna *et al*.,

2011; Anuradha *et al*., 2012). Similar approaches have been successful in wheat (Tiwari *et al*., 2009; Zhao *et al*., 2009; Liu *et al*., 2014), potatoes (Brown *et al*., 2006; Van Eck *et al*., 2007), and sweet potatoes (Oki *et al*., 2002; Low *et al*., 2007; Teow *et al*., 2007). All the above mentioned efforts toward bio-fortifications stem from the fact that staple food crops will improve the nutritional levels of local communities that rely on this crops, and it is a cheap option to take compared to food supplementation.

9.6 Practical implications of the results from this study

The choice of the genotyping and phenotyping methods used in this research was aimed at meeting the interest of the different stakeholders, most importantly, farmers, consumers, breeders, scientists and conservationists. First of all, by comparing the two approaches, molecular method provides accurate results that are less affected by environmental conditions and are used commonly by scientists, breeders and conservationists; however, they are expensive to undertake and the results are difficult to interpret by farmers. The agro-morphological method uses morphological characters with which farmers are well familiar and is less expensive to implement; however, plant characters are affected by environmental conditions that may affect the accuracy of the results. Therefore, using these two methods concurrently in this study has provided accurate and adequate information that is useful to common-bean farmers, consumers, breeders, scientists, conservationists, and partner organisations that will all benefit from the results to improve common-bean production, utilisation and consumption in Zambia. The breeders and scientists will benefit from both genotyping and phenotyping results, whereas other stakeholders (farmers, consumers, input dealers, traders) will benefit from the phenotyping data only as it is commonly used in the participatory plant breeding.

The information generated from this study can be useful in seed registration of these Zambian landraces, hence promoting their production, marketing and utilisation (including conservation) at local, national and regional levels. The results of this study have shown that by keeping the landraces as seed admixtures their genetic composition does not alter from season to season. This agrees with earlier observation that alteration of genetic materials require from hundreds to thousands of years for self-pollinating species in order for the genetic composition to be altered (Beebe *et al*., 1997). This same information is very useful to conservationists in maintaining their high genetic diversity of seeds and well-structured populations for future breeding work. Additionally, these results mean the seeds of these landraces can be exchanged with other

neighbouring countries to be used in breeding to address the abiotic, biotic and nutritional challenges that face the common-bean eating communities.

Safeguarding the nutritional and health status of consumers/farmers and the production environments is another key area of practical importance from this study. This study has highlighted that there is a high genetic and nutritional (iron and zinc) diversity alongside a wider production environments for these landraces of common bean. This places these landraces in a good position in future breeding as parental lines for nutrient enhancement and wider environmental adaptations. This will therefore guarantee the nutritional, health and production environments of the consumers/producers that constantly rely on these landraces and their progenies now and in the future. This will also contribute towards improving the livelihoods of the farming communities involved in growing these landraces.

9.7 Recommendations on future use of these landraces of common bean from Zambia

1. There is need to undertake genetic fingerprinting of these landraces of common bean from Zambia. The high levels of seed admixture exhibited by common-bean landraces indicates that beans from both genepools being grown together as well as commercial varieties. Additionally, there was a controversy regarding the origin of Lundazi as landrace in Zambia or a commercial variety in Malawi. Fingerprinting would reduce duplication that may be arise from the registration these landraces as well as solving the controversy surrounding the origin of Lundazi, in addition to knowing the contribution from neighbouring countries to the genetic diversity and population structure of these Zambian common bean landraces. The entire results presented here concentrated on characterisation, diversity and population structure assessment, and mineral content determination, hence fingerprinting could help with the precise identity of these landraces.
2. Secondly, there is need for specific studies that target the use of the different races of common beans as test genotypes. The molecular and agro-morphological methods predicted different number of sub-populations (7 and 12 respectively) among these landraces from Zambia, and that the Mesoamerican genepool dominates. These proposed studies would provide finer differentiation of the populations through giving answers to questions such as, which race dominates in common-beans of Zambia?

3. Additionally, there is need to study the rate of gene flow in common-bean landraces that are maintained as seed admixture to establish the effects of population size and distance required for an asymmetrical gene flow model. Of particular interest would be to study gene flow from landrace to commercial variety, and vice versa. Additionally, there would be the need to compare the rate of gene flow between bush plant types and climbers. Lastly, in would be interesting and useful to study relative rates of gene flow within controlled environment (glasshouse) and an open field in order to provide clues about the role of pollinators in common-bean pollination and fertilisation as well as to uncover the sources and modes of gene flow among common bean varieties that is currently very limited.
4. There is need to start a multi-locational trial of F2 segregating populations for nutritional contents since the inheritance of iron and zinc have been reported to have no maternal effect. Multi-locational trials of segregating populations are aimed at reducing the time taken in performing backcrossing which will allow for generation of new varieties that are well adapted to field environments. African governments, including Zambia, should also begin to consider conditional releases of crop varieties based on regional or provincial needs. That is, releasing new varieties that are specific to growing environmental conditions and the need of the communities growing such varieties, thus, different crop varieties in different regions and or provinces hence promoting genetic diversity of the crops in questions.
5. Finally, there is an urgent need to standardise the materials and methods as well as the reporting units for nutritional studies in order to ease comparison of results of germplasm from different parts of the world so as to promote germplasm exchange to improve the nutritional status of these staple crops. During this research, it became apparent that there are a lot of differences in the procedure used to determine these minerals in food crops. For example, in the use of Atomic Absorption Spectroscopy (AAS) that this study adopted, differences exist right from acid digestion of the ground powdered samples to presentations of results. A case in point, ElMaki *et al*. (2007) and Gouveia *et al*. (2014) used hydrochloric acid (HCl) to digest their samples, whereas Moraghan and Grafton (2001), Tryphone and Nchinbi-Msolla (2010), Akond *et al*. (2011), and Alzahrani *et al*. (2017) used nitric acid (HNO_3) to digest their samples. Even within the nitric acid digestions, there are still the internal differences such as digestion with nitric acid followed by perchloric acid ($HCLO_4$) or the two acids are mixed together during the digestion (Moraghan and Grafton, 2001), and the digestion with HNO_3 followed by

hydrogen peroxide (H_2O_2) as described in Tryphone and Nchinbi-Msolla (2010), and Akond *et al*. (2011). For reproducibility of results, this study adopted the acid digestion described by Alzahrani *et al*. (2017) where nitric acid digestion was followed with an incubation at 50^0C for 1 hour and cooled down to room temperature before filtration. Again, when it comes to presentation of results, several units are used such as parts ppm, µg/g, mg/100g, mg/Kg as in the above-mentioned papers that complicate comparison of results on nutritional/mineral contents from different geographic regions.

9.8 Conclusions

This research has shown that there is a high genetic diversity, a well-structured populations and varying levels of the mineral concentrations in these landraces from Zambia. Further results have showed that Lusaka yellow and Lundazi landraces are dominated by the Andean genepool, whereas Mbala mixture and Solwezi landraces are dominated by the Mesoamerican genepool. However, over all, Mesoamerican beans dominates in Zambia. This research has demonstrated further the local adaptations of these landraces under the different environments under which they are grown in Chapter 4 in terms of yields. Therefore, participatory plant breeding that involved the use of farmers' local grains in the breeding of new varieties with different stakeholders in the bean value chain is an appropriate option for promoting the utilisation of these landraces, and the new varieties that can come from them. Thorough characterisation of these landraces based on genepools, genetic diversity, population structure and minerals concentration presented here, the bean breeders of ZARI and UNZA can now rely upon these data for breeding work within the Zambian context. The low rates of gene flow over the three growing seasons with values that are not significantly different from each other can provide vital information for the common bean seed conservationists and landrace seed registration process as these landraces can be kept for long period before a change in their genetic composition. Seed registration in particular will empower the farmers that are involved in the growing of these landraces, and provide markets for the produced landraces, in addition to increasing their utilisation. Finally, as guided by the nutritional composition of the seeds from these landraces based on seed colours, it is apparent that the individual seed under each landraces based on the dominant seed colours can be introduced into breeding programmes. For example, under Lusaka yellow (yellow seed colour), Lundazi (red and maroon seed colours), Mbala mixture (yellow, maroon and grey colours), and Solwezi (brown and grey seed colours) can be used to breed for enhanced mineral contents in the Zambian commercial varieties of beans. Additionally, CIAT reference line of

G5773 (black seed colour) and G4494 (red mottled seed colour with light pink flower) can provide breeding materials for both the nutrient composition and wider environmental conditions.

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Annexes

Annex 1 Common bean SSR markers that selected and screened using the DNA samples from the Zambian landraces. The screening was conducted using 9 individuals of each landrace at three different annealing temperatures. The optimal temperature is the one reported here as Ta optimised. Number of alleles for each SSR marker were also considered for a primer to be selected as discriminatory to be used the main study.

SN	Primer	Sequences	Length	Product size	Published Ta	Optimized Ta
01	BMd01*	**F**: CAAATCGCAACACCTCACAA	20	165	50^{u}, 56^{k}, 47^{m}	54
		R: GTCGGAGCCATCATCTGTTT	20			
02	BMd03*	**F**: TGTTTCTTCCTTATGGTTAGGTTG	24	223	47^{m}	57
		R:GTATCCTCCGATCAAATTCACCT	23			
03	BMd04*	**F**: CTCCACTACCCCAAACAGTACC	22	145	47^{m}	58
		R: TTTGAGGAAATGGTTGTTTGGT	22			
04	BMd07*	**F**: GGATATGGTGGTGATCAAGGA	21	166	47^{m}	57
		R: CATACCCAATGCCATGTTCTC	21			
05	BMd08*	**F**: TTCATCCTCTCTCCCGAACTT	21	176	47^{m}	58
		R: CTTTTGTGGCTGAGACATGGT	21			
06	BMd10*	**F**: GCTCACGTACGAGTTGAATCTCAG	24	139	56^{k}, 47^{m}	61
		R: ATCTGAGAGCAGCGACATGGTAG	23			
07	BMd11*	**F**: GCTCAACATTCCAGAGGCTAA	21	161	47^{m}	56.5
		R: TCAAACCTACATAAATAAAACAAAACA	27			
08	BMd18*	**F**: AAAGTTGGACGCACTGTGATT	21	156	47^{m}	59
		R: TCGTGAGGTAGGAGTTTGGTG	21			
09	BMd20*	**F**: GTTGCCACCGGTGATAATCT	20	123	50^{u}, 56^{k}, 47^{m}	58
		R: GTGAGGCAAGAAGCCTTCAA	20			
10	BMd28*	**F**: TGCATCAACTTTAGGAGCTTG	21	151	47^{m}	57
		R: TCTTGTCTTATCAGCAGGTGGA	22			
11	BMd32*	**F**: ACACCCTTCATCTCCCTCAT	20	111	47^{m}	57

		R: ACCCATGTTGGATGTTGGAT	20			
12	BMd47*	**F**: ACCTGGTCCCTCAAACCAAT	20	150	47^{m}	57
		R: CAATGGAGCACCAAAGATCA	20			
13	BMd50*	**F**: TGGTGAGAGAAGGACAATAGCA	22	124	47^{m}	58
		R: GCCGCTTGTGACGTTTATTT	20			
14	BMd53*	**F**: TGCTGACCAAGGAAATTCAG	20	105	50^{u}, 56^{k}, 47^{m}	56
		R: GGAGGAGGCTTAAGCACAAA	20			
15	Pv-ag001*	**F**: CAATCCTCTCTCTCTCATTTCCAATC	26	157	47^{m}	60
		R: GACCTTGAAGTCGGTGTCGTTT	22			
16	Pv-at001*	**F**: GGGAGGGTAGGGAAGCAGTG	20	170	47^{m}	60
		R: GCGAACCACGTTCATGAATGA	21			
17	Pv-at007*	**F**: GAAGAGTTGCAGATTGAGGT	20	149	56^{k}, 47^{m}, 49^{y}	54
		R: TTCTACCAGGCAAATATTGAG	21			
18	BMd33*	**F**: TACGCTGTGATGCATGGTTT	20	110	47^{m}	58
		R: CCTGAAAGTGCAGAGTGGTG	20			
19	BM137**	**F**: GGCTTACTCACTGTACGCACG	21	155	47^{m}	60
		R: CCGTATCCGAGCACCGTAAC	20			
20	BM156**	**F**: CTTGTTCCACCTCCCATCATAGC	23	266	50^{u}, 47^{m}	61
		R: TGCTTGCATCTCAGCCAGAATC	22			
21	BM161**	**F**: TGCAAAGGGTTGAAAGTTGAGAG	23	185	47^{m}	NA
		R: TTCCAATGCACCAGACATTCC	21			
22	BM170**	**F**: AGCCAGGTGCAAGACCTTAG	20	179	47^{m}	NA
		R: AGATAGGGAGCTGGTGGTAGC	21			
23	BM175**	**F**: CAACAGTTAAAGGTCGTCAAATT	23	170	50^{u}, 47^{m}	56
		R: CCACTCTTAGCATCAACTGGA	21			
24	BM183**	**F**: CTCAAATCTATTCACTGGTCAGC	23	149	47^{m}	58
		R: TCTTACAGCCTTGCAGACATC	21			
25	BM187**	**F**: TTTCTCCAACTCACTCCTTTCC	22	191	47^{m}	57
		R: TGTGTTTGTGTTCCGAATTATGA	23			
26	BM200**	**F**: TGGTGGTTGTTATGGGAGAAG	21	221	47^{m}	58
		R: ATTTGTCTCTGTCTATTCCTTCCAC	25			

27	BM210**	**F**: ACCACTGCAATCCTCATCTTTG	22	166	47[m]	58
		R: CCCTCATCCTCCATTCTTATCG	22			
28	BM211*	**F**: ATACCCACATGCACAAGTTTGG	22	186	47[m]	58
		R: CCACCATGTGCTCATGAAGAT	21			
29	PV-BR5*	**F**: ATTAGACGCTGATGACAGAG	20	195	56[b]	55
		R: AGCAGAATCCTTTGAGTGTG	20			
30	PV-BR18*	**F**: GTTCTGCTTGCAGCATACC	19	198	46[b]	NA
		R: AGAAACACAATCGGAAGAG	19			
31	PV-BR20*	**F**: TGAGAAAGTTGATGGGATTG	20	197	56[b]	55
		R: TACGCTGTTGAAGGCTCTAC	20			
32	PV-BR21*	**F**: GAAGAACCGCAAGTAGAGAAGT	22	229	56[b]	NA
		R: TAACATCAGACGCCGACGA	19			
33	PV-BR25*	**F**: GAGCTTCTCCGTCCTGTGT	19	158	56[b]	56
		R: CGAACTGAATCAGAAAGGAA	20			
34	Pv-ctt001*	**F**: GAGGGTGTTTCACTATTGTCACTGC	25	152	50[u], 48[y]	56.5
		R: TTCATGGATGGTGGAGGAACAG	22			
35	Pv-ag003*	**F**:36 TCACGTACGAGTTGAATCTCAGGAT	25	164	56[k], 50[u], 47[y]	56.5
		R: G37GTGTCGGAGAGGTTAAGGTTG	22			
36	Pv-at004*	**F**: AAT38CTGCCGAGAGTGGTCCTGC	22	163	47[y]	NA
		R: GATT39GAAATATCAAAGAGAATTGTTACC	28			
37	Pv-gaat002*	**F**: ACCT40AGAGCCTAATCCTTCTGCGT	24	139	49[y]	56.5
		R: GAATGTGAATATCAGAAAGCAAATGG	26			
38	Pv-at006*	**F**: CCGTTGCCTGTATTTCCCCAT	21	132	50[y]	56.5
		R: CGTGTGAAGTCATCTGGAGTGGTC	24			
39	Pv-at002*	**F**: GTTTCTTCCTTATGGTTAGGTTGTTTG	27	244	49[y]	56.5
		R: TCACGTTATCACCAGCATCGTAGTA	25			

40	Pv-tttc001*	**F**: TTTACGCACCGCAGCACCAC	20	161	49[y]	50
		R: TGGACTCATAGAGGCGCAGAAAG	23			
41	Pv-atgc002*	**F**: AGCTTTCACACTATGACACCACTGG	25	144	50[u], 49[y]	59
		R: TGCGACATGAGAGAAAGACACGG	23			
42	Pv-gat001*	**F**: AGTGGTGTGGATGCTGTTGTT	21	193	47[y]	56.5
		R: GGCGCTGAGATCAGTAGGAG	20			
53	Pv-cct001*	**F**: CATTCTTCCGTATCCCCTGA	20	137, 256, 218, 571	50[y]	NA
		R: ATGCAGCACCACCAAATACA	20			
44	C9**	**F**:ACAGAGACGAGTGCGTGAGAGTTAG	25	445–470	57^	57
		R:AAAGACAGTTCTAGGAAGAACCGTC	25			
45	C33**	**F**: CTCTTTCTGCTTCCTTTCTACGC	23	536–565	59^	59
		R: TTCTTCACAGTCAAGGGAGTAGAAG	25			
46	C106**	**F**:TTGCAGGTAGCAGGTTGT	18	383–431	57^	NA
		R:CAGACAGATAGATAGAGACGG	21			
47	C119**	**F**: CCACCATTGCTCTCAGTGTTA	21	251–292	57^	57
		R: TAGATGTGTGTTTGTGTTCCG	21			
48	C132**	**F**: CAGTGGTTATTCTGGGGATT	20	477–502	58^	58
		R: GGTTGTTTATGGCAGTAGCA	20			
49	C136**	**F**:GTAAAAGTCTCCTTCTACTTTCCCC	25	272–315	60^	NA
		F:GTAAAAGTCTCCTTCTACTTTCCCC	25			
50	G10**	**F**: TCTTCTGTCCATCCCTCCATACT	23	220–273	60^	NA
		R: GATTGGTGGAAATCGACTTGTCT	24			

[u]Published by **Burle *et al.* 2010** with the Brazilian common bean landraces, ^published by **Wang *et al.* 2012** with DNA extracted from the seeds of common bean, [b]published by **Buso *et al.* 2006** with colonies from a single plant of the common bean (*Phaseolus vulgaris*, Perola cultivar), [k]published by **Kwak and Gepts, 2009** with common bean from the two genepools, [y]published by **Yu *et al*. 2000** with the recombinant inbred population of common bean, [m]published by **Blair *et al.* 2003** and **2006** with common bean from CIAT collection centre, NA represents SSR primers that did not amplify any product during optimisation, *Gene based markers (from coding sequences), **Genomic markers (from non-coding sequences).

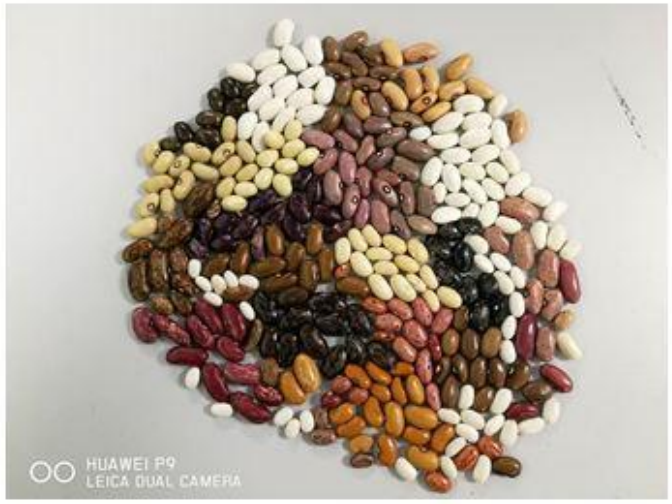
Annex 2 Final 28 SSRs marker selected for use in the assessment of genetic diversity and population structure of the common bean landraces from Zambia

Serial No	Primer Name	FWD sequences	Expected product sizes by different studies					Optimised lab size	Ta optimised
			Wang *et al.* 2012	Blair *et al.* 2003	Blair *et al.* 2006a	Buso *et al.* 2006	Yu *et al.* 2000		
01	BMd01	F: CAAATCGCAACACCTCACAA R: GTCGGAGCCATCATCGTTTT	-	165	124-134	-	-	170-200	55
02	BMd53	F: TGCTGACCAAGGAAATTCAG R: GGAGGAGGCTTAAGCACAAA	-	105	108-112	-	-	105	56
03	BMd20	F: GTTGCCACCGGTGATAATCT R: GTGAGGCAAGAAGCCTTCAA	-	-	168-218	-	-	175-190	58
04	BMd28	F: TGCATCAACTTTAGGAGCTTG R: TCTTGTCTTATCAGCAGGTGGA	-	151	196-200	-	-	140-165	57
05	Pv-at001	F: GGGAGGGTAGGGAAGCAGTG R: GCGAACCACGTTCATGAATGA	-	-	170-295	-	115	239	60
06	Pv-at007	F: GAAGAGTTGCAGATTGAGGT R: TTCTACCAGGCAAATATTGAG	-	-	190-216	-	161	400-450	55
07	Pv-at006	F: CCGTTGCCTGTATTTCCCCAT R: CGTGTGAAGTCATCTGGAGTGGTC	-	-	-	-	132	132-160	57
08	BMd32	F: ACACCCTTCATCTCCCTCAT R: ACCCATGTTGGATGTTGGAT	-	150	143-258	-	-	111	57
09	BMd07	F: GGATATGGTGGTGATCAAGGA R: CATACCCAATGCCATGTTCTC	-	166	122-126	-	-	176	57
10	BMd33	F: TACGCTGTGATGCATGGTTT R:CCTGAAAGTGCAGAGTGGTG	-	-	-	-	-	110	58
11	BM137	F: GGCTTACTCACTGTACGCACG R: CCGTATCCGAGCACCGTAAC	-	-	195-203	-	-	122-148	60
12	BM156	F: CTTGTTCCACCTCCCATCATAGC R:TGCTTGCATCTCAGCCAGAATC	-	-	126	-	-	220-280	60
13	BM175	F: CAACAGTTAAAGGTCGTCAAATT R: CCACTCTTAGCATCAACTGGA	-	-	210-275	-	-	150-170	56
14	BM183	F: CTCAAATCTATTCACTGGTCAGC R: TCTTACAGCCTTGCAGACATC	-	-	134-160	-	-	149-180	58
15	BM187	F: TTTCTCCAACTCACTCCTTTCC R: TGTGTTTGTGTTCCGAATTATGA	-	-	150-270	-	-	191-265	57
16	BM200	F: TGGTGGTTGTTATGGGAGAAG R: ATTTGTCTCTGTCTATTCCTTCCAC	-	-	221-295	-	-	221-260	60
17	BM211	F: ATACCCACATGCACAAGTTTGG	-	-	180-237	-	-	180-210	58

		R: CCACCATGTGCTCATGAAGAT							
18	PV-BR25	R: TAACATCAGACGCCGACGA R: GAGCTTCTCCGTCCTGTGT	-	-	-	158	-	158	55
19	C33	F: CTCTTTCTGCTTCCTTTCTACGC R: TTCTTCACAGTCAAGGGAGTAGAAG	536-565	-	-	-	-	135	58
20	C119	F: CCACCATTGCTCTCAGTGTTA R: TAGATGTGTGTTTGTGTTCCG	251-292	-	-	-	-	290-350	57
21	Pv-tttc001	F: TTTACGCACCGCAGCACCAC R: TGGACTCATAGAGGCGCAGAAAG	-	-	143-258	-	161	161	50
22	BMd03	F: TGTTTCTTCCTTATGGTTAGGTTG R: GTATCCTCCGATCAAATTCACCT	-	-	142-146	-	-	223	58
23	BMd18	F: AAAGTTGGACGCACTGTGATT R:TCGTGAGGTAGGAGTTTGGTG	-	-	154-242	-	-	156	59
24	Pv-ag003	F: TCACGTACGAGTTGAATCTCAGGAT R: GGTGTCGGAGAGGTTAAGGTTG	-	-	157-168	-	164	164	56.5
25	Pv-ctt001	F: GAGGGTGTTTCACTATTGTCACTGC R: TTCATGGATGGTGGAGGAACAG	-	-	152-172	-	164	152	56.5
26	Pv-atcg002	F: AGCTTTCACACTATGACACCACTGG R: TGCGACATGAGAGAAAGACACGG	-	-	-	-	144	144	59
27	Pv-gat001	F: AGTGGTGTGGATGCTGTTGTT R: GGCGCTGAGATCAGTAGGAG	-	-	-	-	182	193-250	56.5
28	Pv-gaat002	F: ACCTAGAGCCTAATCCTTCTGCGT R: GAATGTGAATATCAGAAAGCAAATGG	-	-	156-166	-	167	139	56.5

The colour of the forward SSR marker represents the colour of the fluorescent dye that was used for that particular SSR marker.

Annex 3 Population structure and overlaps revealed the seed types (colour, shape and size) for the Zambian common bean landraces (Top left: Lusaka yellow beans; Top right: Lundazi beans; Bottom left: Mbala mixture beans; and Bottom right: Solwezi beans).



Annex 4 Molecular markers showing common allelic sizes with frequency greater than 25 percent by populations and growing seasons

Locus		Allele size by population and years																							
		Lusaka Yellow			Lundazi			Mbala Mixture			Solwezi			G9794			G5773			G4494			G14470		
		2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
BMd20		119	119	119	119	120	119	119	119	119	119	119	119	119		128	119	123	123	119	119	119	119	119	119
				120		118	129	128	120	128		118	128										123		
									125	120		120											125		
												128													
BM211		192	191	180	188	187	187	181	180	180	181	181	180	181	181	181	181	181	181	181	181	181	181	181	181
		191	188	190	187	191	180	187	182	181	182	182	181	182	182	182	182	187	187	182	187	187	187	187	187
		188	192	186	191	181	188		187	187	187	187	187	187						187			182		
		187	185	191																			188		
		182	181																						
			182																						
C119		296	296	292	358	292	292	270	292	282	292	282	292	290	270	270	290	270	270	292			270		
		366	292	290	318	318	320	282	270	270	270	292	290	292			292			290			290		
				296	362		270	294	290	292				296			296								
							360										270								
BMd07		173	172	172	173	173	172	169	169	169	169	169	169	169	169	169	169	169	169	169	172	172	169	172	172
		172		169	172	173	169	170	170	172	170	172	172	170			170	170	170	170	173	173	172	173	173
				173	169	169		173	173		172	170		172			172			172			170		
						170		172	172		173	173													
BMd53		106	106	106	106	106	106	103	103	103	103	103	103	103	103	103	103	106	106	103	106	106	106	106	106
			107	103	103	103	103	106	104	106	106	104	107	106			106			106			103		
						107	107		107			106													
						104																			
Pv-BR25		162	163	162	162	162	162	160	160	160	160	160	160	160	160	160	160	162	162	160	162	162	162	162	162
					160	160	163	162	162	162	162	163	162				162			162			160		
BMd28		183	183	183	151	183	183	148	181	171	183	148	171	183	148	148	183	183	183	183	183	183	183	151	151

				148	183	151	151 171	183 149	148 182 177	173 183	148	183 151	183	148 175 194	175	173	179 148			194	151	151	148 149 173	185	185
BMd01		202	202	201	202	202 201	202	201 202	202	201 202	201	201	202 201	201	202	201	201	201	202	201	201	201	201 202	201	201
BM137		111 113	111	112 123 114	111 121	111 117	112 113	117 111	117	112 116 114	117 111	117 111	118 124 119 112	117 122	117	117	117	111 117	111 117	117 111	117 111	111 117	117 111	113 117	113 117
BMd18		154	154	154 213	154	154	155 154 213 221	240 154	214 154 222	221 154 213	154 240 213	154 221	213 154	213 154 240	213 240	213 240	213 240 154	213	213	213 154 240	154	154	213 240 154	154	154
Pv-atgc002		140 153	141	141 153	148 140	141 148	141 153	141 148 153 140	141 153 148	141 153	141 153	141 148	141 153	141 153	153	153	141 148	153	153	141 153	141	141	153 148	141	141
BMd03		105	105	105 106	105	106	107 105	105 224 225	106	106 105	224 105 226 225	106	105 106 224	224 226 105	224 226	224 226	226 224	224 226	224 226	226 224 105	105	105	226 224	105	105
Pv-ag003		163	163	163 164	163 164	163 164	163 164	163 164	164 163	163 164	163 164	163 164	164 163	163 164	164	164	163 164	164	164	163 164	163	163	163 164	163	163
Pv-cttt001		162 154	154 160	160 150 154	160 151	160 151 163	160 150 151	150 157 160 163	173 151 157	150 160 157 173	173 150 157 160	151 157 160	172 150	173 150 160		163	173 150 163	157	157	173 150	160	160	150 157 163 173	150	150
BM33		105	105	106	105	106	105	96	96	96	96	96	96	96	93	93	96	96	96	96	105	105	96	105	105

				96 105		96	97 98 96	105	93 105	106 97	105 93	105	93 105	105			105			105			93	106	106
Pv-tttc001		159	159	159	159	159	159	159	159 160	159 160	159 160	161 160	159 160	159	160	160	159	159	159	159	159	159	159 160	159	159
BMd32		108	109	108	108 109	109 108	108	108 109	109 108	108	108 109	109	109	109	109	109	109	109	109	109	109	109	109 110	109	109
Pv-at007		405 406	406 442 405	405 406 430 442 444	405 406	442 406 404	405 442	443 406 405 442	443	443 405 444	405 406 442 443	444 406	444 443 405				405						405 406		
Pv-gat001		189 194	189	189 194	193 189	193 189	193 189	189 193	193 189	189 255 193	189 193 194	193 189	189 193	189 194	196 195 230	196 195 230	189 194			194 189			189 194 196	194	194
Pv-gaat002		139	139	139	139	139	139	139	139	139 140	139	139	139 140		134 135	134 135		134 135	134 135		139	134 135		139	134 135
Total No.		**31**	**30**	**45**	**36**	**43**	**46**	**49**	**47**	**50**	**49**	**44**	**45**	**39**	**24**	**26**	**38**	**23**	**23**	**36**	**21**	**22**	**48**	**23**	**24**
Overall Total			**106**			**125**			**146**			**138**			**89**			**84**			**79**			**95**	

Annex 5 Comparing the significance of differences between means for parental seed widths, and offspring seed width for the different populations

	Parental Width				Offspring Width			
Populations	**diff**	**Lower**	**Upper**	**padj**	**diff**	**Lower**	**Upper**	**padj**
B-A	-0.05100	-0.81710	0.71510	1.00000	-0.31500	-0.94308	0.31308	0.83131
C-A	-0.20200	-0.96810	0.56410	0.99736	-0.20500	-0.83308	0.42308	0.98734
D-A	-0.00900	-0.77510	0.75710	1.00000	0.05900	-0.56908	0.68708	1.00000
E-A	0.67800	-0.08810	1.44410	0.12932	0.44200	-0.18608	1.07008	0.40999
F-A	0.61700	-0.14910	1.38310	0.22707	0.63600	0.00792	1.26408	**0.04465**
G-A	0.65100	-0.11510	1.41710	0.16762	-0.43700	-1.06508	0.19108	0.42664
H-A	-0.62600	-1.39210	0.14010	0.21008	-0.65900	-1.28708	-0.03092	**0.03184**
I-A	0.19300	-0.57310	0.95910	0.99814	-0.34000	-0.96808	0.28808	0.76003
J-A	0.17900	-0.58710	0.94510	0.99897	-1.30100	-1.92908	-0.67292	**0.00000**
C-B	-0.15100	-0.91710	0.61510	0.99974	0.11000	-0.51808	0.73808	0.99990
D-B	0.04200	-0.72410	0.80810	1.00000	0.37400	-0.25408	1.00208	0.64763
E-B	0.72900	-0.03710	1.49510	0.07599	0.75700	0.12892	1.38508	**0.00655**
F-B	0.66800	-0.09810	1.43410	0.14262	0.95100	0.32292	1.57908	0.00017
G-B	0.70200	-0.06410	1.46810	0.10136	-0.12200	-0.75008	0.50608	0.99977
H-B	-0.57500	-1.34110	0.19110	0.31819	-0.34400	-0.97208	0.28408	0.74759
I-B	0.24400	-0.52210	1.01010	0.98935	-0.02500	-0.65308	0.60308	1.00000
J-B	0.23000	-0.53610	0.99610	0.99303	-0.98600	-1.61408	-0.35792	0.00008
D-C	0.19300	-0.57310	0.95910	0.99814	0.26400	-0.36408	0.89208	0.93491
E-C	0.88000	0.11390	1.64610	**0.01192**	0.64700	0.01892	1.27508	**0.03805**
F-C	0.81900	0.05290	1.58510	**0.02637**	0.84100	0.21292	1.46908	**0.00145**
G-C	0.85300	0.08690	1.61910	**0.01706**	-0.23200	-0.86008	0.39608	0.97080
H-C	-0.42400	-1.19010	0.34210	0.73614	-0.45400	-1.08208	0.17408	0.37117
I-C	0.39500	-0.37110	1.16110	0.80749	-0.13500	-0.76308	0.49308	0.99947
J-C	0.38100	-0.38510	1.14710	0.83812	-1.09600	-1.72408	-0.46792	**0.00001**
E-D	0.68700	-0.07910	1.45310	0.11820	0.38300	-0.24508	1.01108	0.61606

F-D	0.62600	-0.14010	1.39210	0.21008	0.57700	-0.05108	1.20508	0.09948
G-D	0.66000	-0.10610	1.42610	0.15401	-0.49600	-1.12408	0.13208	0.25114
H-D	-0.61700	-1.38310	0.14910	0.22707	-0.71800	-1.34608	-0.08992	0.01261
I-D	0.20200	-0.56410	0.96810	0.99736	-0.39900	-1.02708	0.22908	0.55922
J-D	0.18800	-0.57810	0.95410	0.99848	-1.36000	-1.98808	-0.73192	**0.00000**
F-E	-0.06100	-0.82710	0.70510	1.00000	0.19400	-0.43408	0.82208	0.99144
G-E	-0.02700	-0.79310	0.73910	1.00000	-0.87900	-1.50708	-0.25092	**0.00070**
H-E	-1.30400	-2.07010	-0.53790	**0.00001**	-1.10100	-1.72908	-0.47292	**0.00001**
I-E	-0.48500	-1.25110	0.28110	0.56412	-0.78200	-1.41008	-0.15392	**0.00424**
J-E	-0.49900	-1.26510	0.26710	0.52331	-1.74300	-2.37108	-1.11492	**0.00000**
G-F	0.03400	-0.73210	0.80010	1.00000	-1.07300	-1.70108	-0.44492	**0.00001**
H-F	-1.24300	-2.00910	-0.47690	**0.00004**	-1.29500	-1.92308	-0.66692	**0.00000**
I-F	-0.42400	-1.19010	0.34210	0.73614	-0.97600	-1.60408	-0.34792	**0.00010**
J-F	-0.43800	-1.20410	0.32810	0.69858	-1.93700	-2.56508	-1.30892	**0.00000**
H-G	-1.27700	-2.04310	-0.51090	**0.00002**	-0.22200	-0.85008	0.40608	0.97817
I-G	-0.45800	-1.22410	0.30810	0.64244	0.09700	-0.53108	0.72508	0.99997
J-G	-0.47200	-1.23810	0.29410	0.60203	-0.86400	-1.49208	-0.23592	**0.00093**

Annex 6 Comparing the significance of differences between means for parental versus offspring seed lengths, and parental versus offspring seed widths for the different populations

Populations	Parental seed Length vs offspring seed length				Parental seed width vs offspring seed width			
	Diff	Lower	Upper	p adj	diff	Lower	Upper	p adj
B-A	0.44111	-1.22855	2.11077	0.99721	0.02371	-0.75671	0.80412	1.00000
C-A	0.08552	-1.58414	1.75518	1.00000	-0.15338	-0.93379	0.62703	0.99973
D-A	0.89492	-0.77474	2.56458	0.76673	-0.02299	-0.80341	0.75742	1.00000
E-A	0.36395	-1.30571	2.03361	0.99938	0.57317	-0.20724	1.35359	0.34551
F-A	-0.60688	-2.27654	1.06278	0.97294	0.46616	-0.31425	1.24658	0.63953
G-A	-0.17848	-1.84814	1.49118	1.00000	0.75464	-0.02577	1.53505	0.06656
H-A	-0.78144	-2.45110	0.88822	0.87880	-0.46971	-1.25012	0.31070	0.62955
I-A	-0.81058	-2.48024	0.85908	0.85371	0.27364	-0.50678	1.05405	0.97867
J-A	-1.97447	-3.64413	-0.30481	0.00852	0.48755	-0.29286	1.26796	0.57877
C-B	-0.35560	-2.02526	1.31406	0.99949	-0.17709	-0.95750	0.60333	0.99915
D-B	0.45380	-1.21586	2.12346	0.99655	-0.04670	-0.82711	0.73371	1.00000
E-B	-0.07716	-1.74683	1.59250	1.00000	0.54947	-0.23095	1.32988	0.40592
F-B	-1.04799	-2.71765	0.62167	0.57221	0.44246	-0.33796	1.22287	0.70470
G-B	-0.61959	-2.28925	1.05007	0.96904	0.73093	-0.04948	1.51135	0.08570
H-B	-1.22256	-2.89222	0.44710	0.34979	-0.49342	-1.27383	0.28700	0.56198
I-B	-1.25169	-2.92135	0.41797	0.31710	0.24993	-0.53048	1.03034	0.98855
J-B	-2.41558	-4.08524	-0.74592	**0.00042**	0.46384	-0.31657	1.24426	0.64605
D-C	0.80940	-0.86026	2.47906	0.85478	0.13039	-0.65002	0.91080	0.99993
E-C	0.27843	-1.39123	1.94809	0.99993	0.72656	-0.05386	1.50697	0.08969
F-C	-0.69240	-2.36206	0.97726	0.93840	0.61955	-0.16087	1.39996	0.24257
G-C	-0.26399	-1.93365	1.40567	0.99996	0.90802	0.12761	1.68843	**0.01039**
H-C	-0.86696	-2.53662	0.80270	0.79775	-0.31633	-1.09674	0.46409	0.94626
I-C	-0.89609	-2.56575	0.77357	0.76538	0.42702	-0.35340	1.20743	0.74497
J-C	-2.05999	-3.72965	-0.39033	**0.00493**	0.64093	-0.13948	1.42134	0.20268
E-D	-0.53097	-2.20063	1.13869	0.98911	0.59617	-0.18425	1.37658	0.29175
F-D	-1.50180	-3.17146	0.16786	0.11508	0.48916	-0.29126	1.26957	0.57417
G-D	-1.07339	-2.74305	0.59627	0.53826	0.77763	-0.00278	1.55805	0.05160

H-D	-1.67636	-3.34602	-0.00670	**0.04825**	-0.44672	-1.22713	0.33370	0.69325
I-D	-1.70549	-3.37515	-0.03583	**0.04127**	0.29663	-0.48378	1.07704	0.96391
J-D	-2.86939	-4.53905	-1.19973	**0.00001**	0.51054	-0.26987	1.29095	0.51312
F-E	-0.97083	-2.64049	0.69883	0.67400	-0.10701	-0.88742	0.67340	0.99999
G-E	-0.54242	-2.21208	1.12724	0.98735	0.18147	-0.59895	0.96188	0.99897
H-E	-1.14539	-2.81505	0.52427	0.44383	-1.04288	-1.82330	-0.26247	**0.00156**
I-E	-1.17453	-2.84419	0.49514	0.40720	-0.29954	-1.07995	0.48087	0.96161
J-E	-2.33842	-4.00808	-0.66876	**0.00073**	-0.08562	-0.86604	0.69479	1.00000
G-F	0.42840	-1.24126	2.09807	0.99777	0.28848	-0.49194	1.06889	0.96981
H-F	-0.17456	-1.84422	1.49510	1.00000	-0.93587	-1.71629	-0.15546	**0.00715**
I-F	-0.20370	-1.87336	1.46596	1.00000	-0.19253	-0.97294	0.58788	0.99836
J-F	-1.36759	-3.03725	0.30207	0.20569	0.02139	-0.75903	0.80180	1.00000
H-G	-0.60297	-2.27263	1.06669	0.97406	-1.22435	-2.00476	-0.44394	**0.00009**
I-G	-0.63210	-2.30176	1.03756	0.96481	-0.48100	-1.26142	0.29941	0.59747
J-G	-1.79599	-3.46566	-0.12633	**0.02491**	-0.26709	-1.04750	0.51332	0.98188
I-H	-0.02913	-1.69879	1.64053	1.00000	0.74334	-0.03707	1.52376	0.07517
J-H	-1.19303	-2.86269	0.47663	0.38461	0.95726	0.17685	1.73767	**0.00533**
J-I	-1.16389	-2.83355	0.50577	0.42043	0.21391	-0.56650	0.99433	0.99632